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Prey selection and digestive processing in terrestrial carnivorous mammals

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"You can't start a fire without a spark"

Bruce Springsteen - Dancing in the dark

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List of abbreviations

Ace = acetic acid

ADF = acid detergent fibre

BARF = bone and raw food

BCFA = branched-chain fatty acids

But = butyric acid

C = gut capacity

CF = crude fibre

CI = confidence interval

CTT = colonic transit time

DM = dry matter

E_{prey} = metabolisable energy in prey

FMR = field metabolic rate

FO = frequency of occurrence

GET = gastric emptying time

GMC = giant migrating complexes

GRT = gastric residence time

IMMC = interdigestive migratory myoelectric complex

iM_{prey} = pack corrected prey mass

isoBut = iso-butyric acid

isoVal = iso-valeric acid

KF = kill frequency

M = body mass

MaxRT = maximum retention time

ME = metabolisable energy

MER = maintenance energy requirements

M_{pred} = predator body mass

M_{prey} = prey mass

MRT = mean retention time

NfE = nitrogen free extract

N_{pack} = pack size

OLS = ordinary least squares

PGLS = phylogenetic generalized least squares

Pro = propionic acid

Q_{pred} = carnivore specific maintenance requirements

rFO = relative frequency of occurrence

SBTT = small bowel transit time

SCFA = short-chain fatty acids

SD = standard deviation

SEM = standard error of the mean

$T_{1/2}$-GET = gastric half emptying-time

TDF = total dietary fibre

TTT = total transit time

Val = valeric acid

General introduction

1. What makes a carnivore?

1.1 Carnivore diversity: a dietary perspective

The mammalian order of Carnivora harbours a great diversity of species. Taxonomic classification renders 281 species included in 128 genera and 16 families (Wilson and Reeder, 2005) (Table 1). The order is characterised by a significant variation in terms of morphology, ecology and behaviour (Gittleman, 1989). Body size can range from a species as small as the least weasel (*Mustela nivalis*) that weighs ca. 50 grams to a gigantic southern elephant seal (*Mirounga leonina*) of more than 3500 kg (Nowak, 1999). Carnivores inhabit various habitats from aquatic terrains to terrestrial environments such as grasslands (e.g. Indian fox, *Vulpes bengalensis*) (Vanak and Gompper, 2007), deserts (e.g. fennec fox, *Vulpes zerda*) (Gittleman, 1989), forests (e.g. pine marten, *Martes martes*) (Storch et al., 1990) and many more. If we ought to give one example of behavioural differences between carnivores, carnivore species can be generally subdivided in solitary (e.g. Eurasian lynx, *Lynx lynx*) (Ratkiewicz et al., 2014) versus social predators (e.g. African wild dog, *Lycaon pictus*) (Creel and Creel, 1995).

When it comes to feeding or dietary habits, classification is not as straightforward as one would expect. Although 'carnivore' literally means 'meat eater', not all species can be as easily regarded as just 'eating meat'. One of the most pronounced examples is the giant panda (*Ailuropoda melanoleuca*) that is taxonomically regarded as a carnivore but thrives on a bamboo-dominated diet, i.e. a completely herbivorous diet (Krause et al., 2008; Xue et al., 2015). Numerous other carnivores rely on an omnivorous (e.g. brown bear, *Ursus arctos*) (Elfström et al., 2014), insectivorous (e.g. aardwolf, *Proteles cristatus*) (Wilman et al., 2014) or even frugivorous diet (e.g. kinkajou, *Potos flavus*) (Julien-Laferrière, 1999). Only members of the felid family and some species of the mustelid family (e.g. least weasel, *M. nivalis*) (King, 1980) are considered strictly carnivorous although recent evidence suggests that wolves (*Canis lupus*, Canidae), that are often referred to as omnivores, are of a true carnivorous kind with only negligible consumption of plant material (Bosch et al., 2015). However, a specific dietary composition can be affected by food availability which in turn can be impacted by seasonal and geographical conditions (Fuller and Sievert, 2001; Hill and Dunbar, 2002). The yellow-throated marten (*Martes flavigula*) e.g. is known to become an opportunistic frugivore when fruit abundance reaches its temporal maximum

in contrast to an otherwise rodent-dominated diet (Zhou et al., 2011). European wildcats (*Felis silvestris*) exhibit greater diet diversity at lower latitudes (i.e., mediterranean climates) where there is greater prey richness (Lozano et al., 2006). It must be clear that only from considering dietary habits of carnivores, great variation originates at an interspecific as well as intraspecific level, contributing to the great diversity that characterizes the Mammalian order of Carnivora.

Table 1 Family subdivision of the Mammalian order of Carnivora

Carnivore family	Number of species	Species example
Ailuridae	1	Red panda (*Ailurus fulgens*)
Canidae	35	Gray wolf (*Canis lupus*)
Eupleridae	8	Fossa (*Cryptoprocta ferox*)
Felidae	37	Lion (*Panthera leo*)
Herpestidae	34	Water mongoose (*Atilax paludinosus*)
Hyaenidae	4	Spotted hyaena (*Crocuta crocuta*)
Mephitidae	12	Striped hog-nosed skunk (*Conepatus semistriatus*)
Mustelidae	57	Pine marten (*Martes martes*)
Nandiniidae	1	African palm civet (*Nandinia binotata*)
Odobenidae	1	Walrus (*Odobenus rosmarus)*
Otariidae	16	Antarctic fur seal (*Arctocephalus gazella*)
Phocidae	19	Common seal (*Phoca vitulina*)
Prionodontidae	2	Banded linsang (*Prionodon linsang*)
Procyonidae	12	Common raccoon (*Procyon lotor*)
Ursidae	8	Brown bear (*Ursus arctos*)
Viverridae	34	Common genet (*Genetta genetta*)

1.2 Digestive physiology of terrestrial carnivores

Current knowledge on carnivore digestive physiology stems from an extensive amount of research conducted with captive non-domestic and domestic carnivores (i.e. domestic dog (*Canis familiaris*) and cat (*Felis catus*)) (e.g. NRC (2006), Stevens and Hume (1995)). Carnivores are typically referred to as having a simple digestive tract because of a highly digestible natural diet compared to other feeding types (Fig. 1) (Stevens and Hume, 1995) and different taxonomic groups seem to share similarities in nutrient digestibility (Clauss et al., 2010). Although carnivore species share certain digestive characteristics, it appears hard to put forward one carnivore digestive prototype since diversity still occurs among carnivores at different levels of digestive physiology.

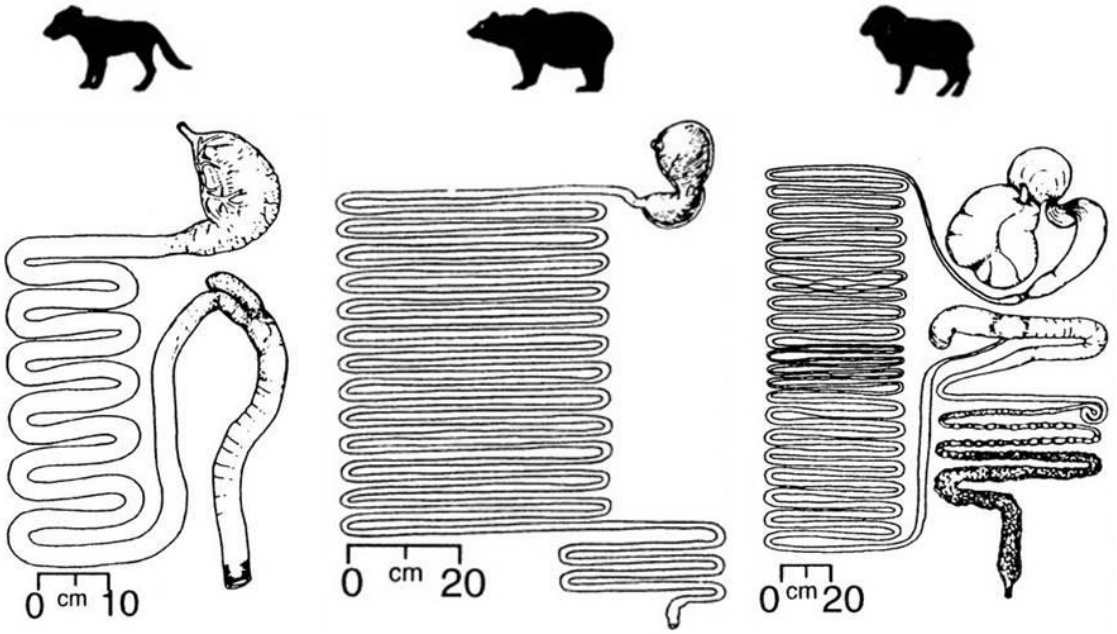

Fig. 1 Gastrointestinal tract of a carnivorous, omnivorous and herbivorous feeder
Left figure: carnivorous feeder, the domestic dog (*Canis familiaris*) with bodylength 90 cm; middle figure: omnivorous feeder, the black bear (*Ursus americanus*) with bodylength 125 cm; right figure: herbivorous feeder, sheep (*Ovis aries*) with bodylength 110 cm (From Stevens and Hume (1995))

1.2.1 *Morphometry, allometry and transit in the gastrointestinal tract*

The carnivore stomach is of a simple nature without any diverticula (Stevens and Hume, 1995; Hume, 2002) and with the potential in some species, e.g. the dog (*C. familiaris*), to expand considerably (Hume, 2002; Bosch et al., 2015). The latter probably originated in wild counterparts in order to accumulate excessive amounts of prey (Hume, 2002). Wolves *(C. lupus)* are able to ingest up to 22 % of their own body weight (Stahler et al., 2006). Tigers *(Panthera tigris)* and lions *(Panthera leo)* can eat up to one fifth of their own bodyweight in a very short period of time (Schaller, 1967; Bertram, 1975). Spotted hyenas *(Crocuta crocuta)* are able to eat one third of their bodyweight in one eating session (Kruuk, 1972). Such large meals require enough gastric capacity and extension and indeed, Bertram (1975) noticed the swollen abdomen of lions after ingesting large amounts of food in a few hours and classified this gastric extension on a scale from

one to five. A similar scaling (belly depth to body length ratio) exists for African wild dogs to assess meat consumed (Potgieter and Davies-Mostert, 2012).

The intestinal tract of carnivores is relatively short with the longest relative lengths measured in mustelids (McGrosky et al., 2016). The small intestine or midgut is short but is perceived as the dominant feature of the carnivore gut where most of the enzymatic digestion takes place (Hume, 2002). The large intestine or hindgut (i.e., colon, caecum and rectum) is comparatively short and simple (Stevens and Hume, 1995). The colon tends to be wide and unsacculated (Hume, 2002). As for the caecum, not all carnivore species possess a caecum which even further simplifies the intestinal tract (Fig. 2) (Mitchell, 1903-6; Kostanecki, 1926; McGrosky et al., 2016). Typically, ursids, mustelids and procyonids and species such as the binturong (*Arctictis binturong*) lack a caecum (McGrosky et al., 2016). For those that possess a caecum, morphological outturn can differ from e.g. a coiled appendage in the dog (*C. familiaris*) to not as coiled in the domestic cat (*F. catus*) (Stevens and Hume, 1995). Omnivorous and herbivorous feeders within the Order of Carnivora show similarities with strict carnivorous feeders, although again some diversity exists. The aardwolf for instance (*P. cristatus;* insectivorous feeder) has a similar digestive tract as the domestic cat and dog (Anderson et al., 1992; Stevens and Hume, 1995) with a caecum that resembles the dog's caecum but is relatively small compared to hyaenids (Anderson et al., 1992). The omnivorous raccoon (*Procyon lotor*) has a relatively longer small intestine than dogs and cats but has a shorter hindgut without caecum (Stevens and Hume, 1995). A complete herbivorous carnivore such as the giant panda (*A. melanoleuca*) has similarly a simple hindgut with no caecum (Dierenfeld et al., 1982; Stevens and Hume, 1995) which infers that a caecum might not be a prerequisite for omnivorous or herbivorous feeders (McGrosky et al., 2016).

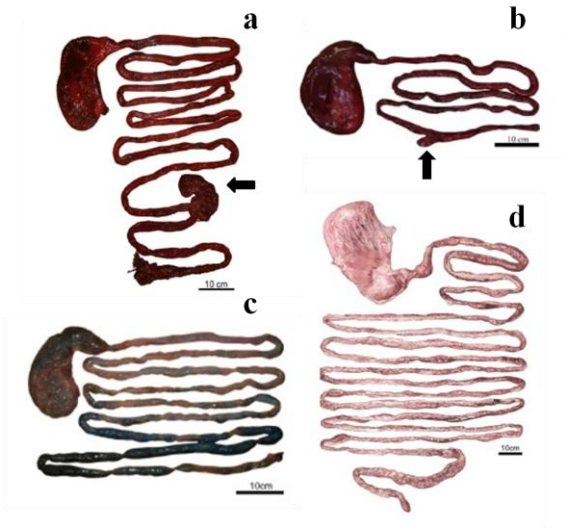

Fig. 2 Gastrointestinal tracts of carnivore species with and without caeca Figure a: the Mongolian wolf (*Canis lupus chango*); figure b: the Canadian lynx (*Lynx canadensis*); figure c: the ferret (*Mustela putorius*); figure d: the brown bear (*Ursus arctos*); black arrows indicate the caecum (Adapted from McGrosky et al. (2016))

Studying the movement of digesta through the gastrointestinal tract connects physiological gut motility and the type of diet (physical and nutritional) provided to the animal (Clemens and Stevens, 1980; Warner, 1981). Typically, the rate at which digesta move through the gastrointestinal tract will be optimal when it matches the rate of feeding, digestion and absorption. The latter is necessary to ensure maximal conversion of the ingested food in as short as possible period and small as possible gut volume (Clemens and Stevens, 1980; Penry and Jumars, 1987; Stevens and Hume, 1995). In general, if one assumes the highly digestible 'meat diet' of carnivores, i.e. high quality foods, this implies a high digestion rate and consequently short digesta retention times compared to other feeding types (Sibly, 1981; Hume, 1989). However, there is no such thing as one typical retention time in carnivores and comparison between species or between different feeding types are constrained by different physical and nutritional characteristics of the diet (Hogan and Weston, 1969; Hintz et al., 1971). For instance, large dietary volumes are known to slow down gastric emptying in dogs (Gupta and Robinson, 1995; Lin, 1996). Meals with a high energy density or with a high fat content can similarly slow down gastric emptying (Meyer et al., 1994; Wyse et al., 2001). Again, in dogs, the inclusion of plant-derived insoluble fibre in the diet can affect transit time: the inclusion of cellulose in a canine diet decreases total transit time

(Burrows et al., 1982) and the addition of 10 % insoluble fibre (sugarcane fibre) in a dog's diet can delay gastric emptying and colonic filling time (Pedreira et al., 2013).

Additionally, digesta retention time as measured by the fecal excretion of marker substances is particularly constrained in its validity in carnivores: the highly digestible diet results in a comparatively low amount of faeces that the animal can afford to not defecate instantaneously, but carry around in its rectum until a behaviourally suitable situation, e.g. for scent marking, presents itself (Parker, 2010). This is in contrast to herbivores that mostly have a more continuous flow of digesta through and hence of faeces out of their digestive tract (Penry and Jumars, 1987).

1.2.2 *Digestion of nutrients and metabolic adaptations in obligate carnivores*

After a diet is ingested it will go through a series of mechanical, chemical and microbial events which can be simply summarized as the process of enzymatic digestion in the upper gut, fermentation of enzymatically undigested components in the lower gut and the absorption of processed dietary components after which they are metabolised in the animal's body (Stevens and Hume, 1995; McDonald et al., 2011). The principal nutrients found in animal diets that are to be digested are carbohydrates, lipids, proteins and nucleic acids (McDonald et al., 2011). The archetype of a carnivorous diet, i.e. animal tissue, is protein-rich and low in carbohydrates, which has led to certain metabolic adaptations in some obligate carnivores (Hume, 2002). The wildcat (*F. silvestris*) is known as a strict, obligate carnivore consuming whole prey, which has led to metabolic adaptations (= idiosyncrasies) still present in domestic cats (MacDonald et al., 1984; Morris, 2002). A summation of metabolic adaptations in domestic cats is given in Table 2. Some mustelids, i.e. the mink (*Mustela lutreola*) and ferret (*Mustela putorius*), which are also perceived as strictly carnivorous, share the same pecularities in enzyme activity for *de novo* synthesis of arginine (Leoschke and Elvehjem, 1959; Deshmukh and Shope, 1983). Lions (*P. leo*) are similarly incapable of synthesising arachidonic acid from linoleic acid (Rivers et al., 1976). Domestic dogs on the contrary do not share the same metabolic adaptations as seen in domestic cats. They are capable of synthesising essentiel nutrients such as arginine, taurine, niacin and arachidonic acid and to down-regulate catabolic amino acid enzymes on low dietary protein diets (MacDonald et al., 1984; Legrand-Defretin, 1994). Therefore, dogs seem to resemble omnivorous species such as pigs (Baker and Speer, 1983) and rats (Harper, 1965). However, recent evidence suggests that the dog is not to be perceived as an omnivore but is of a carnivorous kind as is his wild ancestor the

wolf (Axelsson et al., 2013; Bosch et al., 2015). Bosch et al. (2015) launched the idea that the metabolic differences observed between carnivore species might be caused by differences in feeding strategies in the wild. The typical feast and famine regime (i.e. periods of prey, hence food abundance alternated with periods of famine) to which wolves are often submitted contrasts with the frequent feeding style with regular nutrient intake of cats. Famine periods might have caused a necessity to decrease metabolic losses and preserve the ability to synthesise essentiel nutrients whereas the regular feeding style might have relaxed selection pressure against certain metabolic pathways (see section 2.4). In addition, recent evidence shows that during the domestication of dogs, three genes involved in starch digestion and glucose uptake were subject to selective pressure, suggesting that the domestic dog adapted to starch-rich diets that were common during domestication (see below) (Axelsson et al., 2013). Hence, the ability of dogs to thrive on starch-rich diets does not stem from the so thought omnivorous nature of the wolf but from digestive adaptations that occurred during domestication.

Table 2 Digestive and metabolic adaptations of domestic cats

Metabolic adaptation	Enzymes/Receptor	Reference
Inability to down-regulate catabolic amino acid enzymes when dietary protein is low	Aminotransferases	MacDonald et al. (1984) Morris (2002)
Inability for *de novo* arginine synthesis due to reduced activity of enzymes involved in the intestinal citrulline pathway	Pyrroline-5-carboxylate synthase Ornithine aminotransferase	MacDonald et al. (1984) Morris (2002)
Low activity of enzymes involved in endogenous taurine synthesis	Cystein dioxygenase Cysteinsulfinic acid decarboxylase	MacDonald et al. (1984) Morris (2002)
Inability to synthesise retinol from carotenoids	Carotene dioxygenase	MacDonald et al. (1984) Morris (2002)
Inability to synthesise vitamine D_3 due to high activity of enzymes that catabolyse the precursor	7-dehydrocholesterol	Morris (2002)
Inability to syntesise niacin from tryptophan due to high degradative enzyme activity	Picolinic carboxylase	MacDonald et al. (1984) Morris (2002)
Limited capacity to produce arachidonate from linoleate due to low desaturase activity	Δ6-desaturase Δ8-desaturase	MacDonald et al. (1984) Morris (2002)
Adaptations in sugar and starch metabolism associated with absent or low activity of degradative enzymes	Salivary, pancreatic and intestinal amylase Hepatic glucokinase Hepatic fructokinase	Kienzle (1993a) Kienzle(1993b) Kienzle (1994) Washizu et al. (1999)
Inability to taste the sweetness of sugar due to the absence of a receptor	Tas1R2 receptor	Li et al. (2005)

1.2.3 *Fermentation*

Although characterized by highly digestible diets and a simple large intestine, carnivores do harbour microbial populations in the hindgut in order to ferment nutrients that were enzymatically indigestible or escaped digestion and absorption in the upper tract (Stevens and Hume, 1995; NRC, 2006).

Fermentation is commonly defined as the anaerobic breakdown of carbohydrates and proteins (dietary or endogenous) in the large intestine which renders energy for microbial growth and maintenance (Macfarlane and Gibson, 1995; Wong et al., 2006). Fermentation of dietary carbohydrates such as starch, sugars and fibre (indigestible plant-derived fibre) renders the production of short-chain fatty acids (SCFA) together with the gases CO_2, H_2 and CH_4 (Cummings et al., 1987). Protein fermentation similarly renders SCFA and the branched-chain

fraction (BCFA) of SCFA are typically associated with protein fermentation (Rasmussen et al., 1988; Macfarlane et al., 1992). Next to the production of SCFA, protein fermentation is also associated with the production of putrefactive compounds such as ammonia (NH_3), phenols, indoles, aliphatic amines and sulphur-rich compounds (Cummings and Macfarlane, 1991). Typically SCFA are associated with beneficial gastrointestinal and (after absorption) metabolic effects (e.g. energy for gut epithelial cells and important role in water and Na absorption) (Stevens and Hume, 1998; Wong et al., 2006). Putrefactive compounds, associated with protein fermentation, are known to be detrimental for the health of the host although mainly studied in humans (e.g. association with inflammatory bowel disease in humans) (Matsui et al., 1995; Pedersen et al., 2002; Tuohy et al., 2006). However, some biogenic amines (putrescine, spermidine and spermine) are considered beneficial for cell growth and function in low concentrations (Delzenne et al., 2000).

Typically in herbivorous carnivores (i.e. the giant panda) fermentation will depend on carbohydrate substrates (Xue et al., 2015), in omnivorous carnivores (e.g. the raccoon) on a mixture of carbohydrates and proteins (Clemens and Stevens, 1979), whereas in strict obligate carnivores it is likely that fermentation will depend largely upon proteins. The fermentation of proteins in carnivores has received attention since protein fermentation is perceived as partly detrimental for gut health as explained before, although strict carnivores seem to have adapted to this process. When ingesting whole prey, proteins entering the large intestine can originate from the actual meat and organs (highly enzymatically digestible), since there is always a small fraction that escapes enzymatic digestion, and from compounds such as connective tissues (e.g. collagen), bones, hairs or feathers that are known to be barely enzymatically digestible (Asghar and Henrickson, 1982). The latter compounds will enter the hindgut in a rather unmodified way and can serve as substrates for fermentation (Banta et al., 1978; Macfarlane and Allison, 1986; Depauw et al., 2012; Depauw et al., 2013). This low to non-digestible (glyco)protein-rich matter, such as raw bones, tendons, cartilage, skin, hair or feathers was recently called 'animal fibre' by Depauw et al. (2012, 2013). The authors pointed out the analogies with plant-derived fibre in terms of fermentation potential and fermentative differences between different types of fibre. In their studies with cheetahs (Depauw et al., 2013), the faecal propionic acid, butyric acid, BCFA and putrefactive compounds were higher in cheetahs fed supplemented beef in comparison with cheetahs fed whole rabbit, suggesting more protein fermentation in supplemented beef. Therefore,

it was suggested that differences in fermentation between animal substrates (more fermentable substances such as collagen vs less fermentable compounds such as hair and bones) exist, which was also seen with the *in vitro* fermentation of animal substrates with cheetah faecal inoculum (Depauw et al., 2012). It is possible that indigestible material such as hair and bones acts as a possible bulking agent, forming a physical barrier between substrates and bacteria and filling the large intestine, tempering protein fermentation.

The microbial population and the intestinal production of fermentation metabolites have been studied in several carnivore species, e.g. domestic dogs (Bosch et al., 2008; Bosch et al., 2009; Beloshapka et al., 2012; Panasevich et al., 2015), cheetahs (*Acinonyx jubatus*) (Depauw et al., 2012; Depauw et al., 2013; Becker et al., 2014), domestic cats (Sunvold et al., 1995; Brosey et al., 2000; Ritchie et al., 2008; Kerr et al., 2014a), raccoons (Clemens and Stevens, 1979), the giant panda (Xue et al., 2015), bobcats (*Lynx rufus*), jaguar (*Panthera onca*), tiger (*P. tigris*) (Vester et al., 2008), African wildcat (*Felis lybica*) (Vester et al., 2010b). In general, the gut microbiota within the mammalian order of Carnivora are dominated by the facultative anaerobes *Enterobacteriaceae* and *Enterococcus* (Schwab et al., 2011; Schwab and Gänzle, 2011). The microbial composition in the mammalian gut is mainly, next to gut physiology, shaped by the diet (Ley et al., 2008; Muegge et al., 2011). For instance, when the protein level in diets was varied in domestic cats, the micriobiota profile in cats fed the high protein diet (after eight weeks of adaptation) only showed 40% similarity with the profile of cats fed a moderate protein diet (Lubbs et al., 2009). Similarly, when the comparison between wild ancestors and domestic descendants is made (i.e. the wolf and domestic dog; the wild cat and domestic cat), it is questionable whether the 'domestic' microbiome is still representative for wild counterparts. Today, many carnivorous diets (e.g. canine and feline petfood), but also diets of wild carnivores maintained in captivity, are enriched with plant-derived fibre for its beneficial effects on food intake, appetite and intestinal health (Fahey et al., 2004). However, an obligate carnivore's natural diet barely includes plant fibre. The natural diet of wildcats is characterised by its high protein and low carbohydrate level and thus barely includes plant fibre, whereas the diet of the domestic cat changed substantially to high protein and carbohydrate levels (traditional kibble diets and canned meats) (Plantinga et al., 2011). Similarly, the commercial diets typically fed to dogs have higher carbohydrate (mainly starches) fractions than the natural diet of wolves (Bosch et al., 2015). It seems likely that the inclusion of plant-derived fibre has induced adaptations in the microbiome of domestic carnivores.

Indeed, when studying the microbiome of captive cheetahs fed whole prey, it seemed that significant compositional differences occurred with the microbiome of domestic cats on commercial diets (the latter often being used as a model for exotic felids) (Becker et al., 2014). However, apart from the strong adaptive capacity of microbiota to dietary changes, some evolutionary adaptations have remained preserved in some carnivores. For instance, a new genus of bacteria, *Novospingobium spp*, was recently identified in the hindgut of the domestic cat. This genus is known to use indoles and phenols as fermentation substrate which are typically perceived as detrimental fermentation metabolites from protein fermentation (see above) (Lubbs et al., 2009). The presence of this genus might therefore be an evolutionary adaptation to a natural high protein diet that has preserved throughout domestication. Additionally, the giant panda, which is known to rely on a completely herbivorous diet, has not evolved a gut microbiota adapted to its highly fibrous diet; instead, it shows a typical carnivore-like gut microbiome which contradicts the assumption that adaptation of microbiota to the diet is always obligatory (Xue et al., 2015).

2. The implications of carnivore body size

Within the mammalian order of Carnivora, diversity occurs in all aspects of ecology, behaviour and morphology. The questions "Why are carnivores so diverse?", "How can a certain characteristic be explained?" or "Why is a species as it is?" do not lend themselves to simple answers. In this light, a conversation quoted in Karasov and Diamond (1988) between physiologists and ecologists Martin Cody, Robert MacArthur and Jared M. Diamond while going for a bird walk might offer more perspective in this matter: *"Near a stream they saw a black phoebe (Sayornis nigricans), a species of flycatcher confined to the vicinity of water. To MacArthur's question, "Why do you suppose the black phoebe lives only near water?", Diamond and Cody gave opposite dogmatic responses. Diamond insisted "There must be physiological reasons, like low renal concentrating ability resulting in high water requirements. Physiological factors often determine an animal's ecology." Cody replied equally firmly, "Nonsense. Natural selection makes an animal's physiology adapt to the animal's ecological niche, so that physiology provides nothing more than proximate causes. The ultimate causes must be ecological ones, like food availability near streams or else competition with flycatcher species of drier habitats.""* Many years later, authors agreed that the question "Does physiology constrain ecology?" or vice versa is complex and both can occur, with the time scale at which physiological adaptations take place being a central element.

Although explaining carnivore physiological characteristics and their relation with ecology is a complex issue, one can only try to find regularities in order to elucidate or predict certain biological phenomena. Body size is one of the most obvious features of an animal and can be used in studying the interplay between physiology and ecology, and size-driven diversification (Peters, 1983; Cohen et al., 1993; Cohen, 1994; Carbone et al., 1999; Woodward et al., 2005).

2.1 The basics of body size relationships

Body size relationships typically try to empirically relate body size with biological phenomena, i.e. the animal's characteristics in order to unravel constraints or implications for ecology (Peters, 1983). The latter has been used in paleontology (Gould, 1966; Sander and Clauss, 2008), physiology (Pedley, 1977; Hernot et al., 2005; Blueweiss et al., 2010; Müller et al., 2013; Wilson

et al., 2015), morphology (Thompson, 1961), ecology (Kendeigh et al., 1977; Carbone et al., 2014) and behaviour (Clutton-Brock and Harvey, 1977). Body size relationships are mostly formulated as a power function:

$Y = aM^b$

with Y being the animal's characteristic that is to be predicted, M being the body mass, and *a* and *b* being empirically deducted constants. Initially, data are typically converted to their logarithms in order to simplify and improve graphic and statistical reasoning. Since the variable that is to be predicted (i.e., Y) and M change with different magnitudes or rates, body size power relations are often referred to as allometric relations or as Y scaling to body size (Peters, 1983).

2.2 Body size versus animal physiology

2.2.1 *Metabolic rate and food intake/ingestion versus body size in endothermic mammals*

As simply stated by Peters (1983), what goes into an animal (be it energy or mass) must come out:

Ingestion = somatic or individual growth
+ reproductive growth
+ respiration
+ egestion
+ excretion

The latter formula is referred to as the balanced growth equation with growth factors, respiration, egestion and excretion expressing the rate of energy expenditure per unit time by endothermic animals, i.e. the metabolic rate. Typically, metabolic rate is expressed as a rate of carbon or energy or energy flux (e.g. watt (joules/sec)). The standard metabolic rate expresses the metabolic rate under standard laboratory conditions in animals that are awake, inactive, unexcited, healthy, nonreproductive adults and are in a fasting or postabsorptive state under neutral temperatures. Maximum metabolic rates are measured in trained animals that move at maximum speed. The expension of energy in an animal will occur at a rate somewhere between the standard and maximum metabolic rate and is called the daily energy expenditure or the average daily metabolic rate (Peters, 1983; McNab, 1997).

Metabolic rate has long been recognized as scaling to body size as metabolic rate = aM^b (Kleiber, 1932; Peters, 1983; Nagy et al., 1999; White and Seymour, 2003). For many years, there has been a lot of debate on the constant *b*, whether the value lies around *2/3* or rather *3/4* (White and Seymour, 2005). The scaling exponent of *2/3* stems from the early work of a.o. Rubner (1883) and is based on the 'surface law' of metabolism. Basically the surface law says that the basal metabolism of animals that differ in size is almost proportional to their body surface. The heat that originates from metabolic processes must be dissipated through the body surface, hence, the rate of heat production should be matched to the surface area over which it is released. Knowing that body surface $A \sim M^{2/3}$, it is plausible that the metabolic rate $\sim M^{2/3}$ (White and Seymour, 2005; Hudson et al., 2013). However, later on, empirical work of Kleiber (1932) showed that the metabolic rate did not scale in proportion to body surface area (b = 2/3), but with an exponent significantly greater (b = 3/4). This was further supported by the work of Brody (1945) who found the same exponent for almost the entire body size spectrum of terrestrial mammals and published the well-known mouse-to-elephant curve (Fig. 3). Until today, no consensus is reached on the use of an exponent of 2/3 or 3/4 (White and Seymour, 2003; White and Seymour, 2005) although the use of b = 3/4 has been commonly accepted in comparative physiology for over seven decades and is currently still used. Opponents of the exponent 3/4 (White and Seymour, 2005) have indicated that the increase from a 2/3 to 3/4 exponent simply stems from the inclusion of larger herbivores in empirical datasets since their metabolic rate cannot be measured in a post-absorptive state due to the microbiota inhabiting the gut, even not after a period of fasting. Apart from the discussion on scaling exponents, the fact that a scaling effect exists with body size means that small-bodied animals require more energy and nutrients per day and per unit of bodymass than do large animals (Geist, 1974). However, the scaling of one single measure (here metabolic rate) in itself has no explanatory power. Only when this scaling is compared to the scaling of another factor to body size (such as gut capacity), deductions on size driven diversification can be made (Clauss et al., 2013).

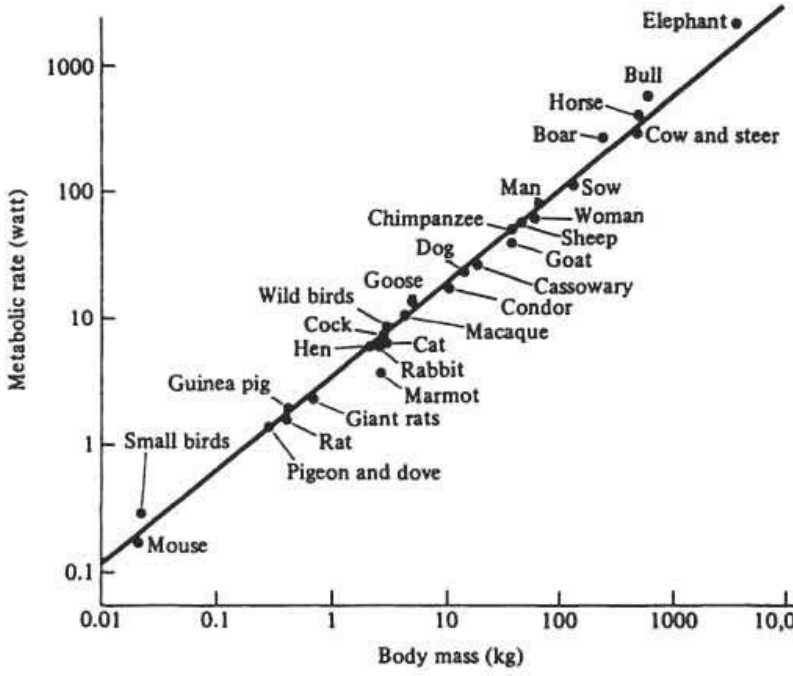

Fig. 3 The mouse-to-elephant curve Metabolic rate is scaled to body mass for several mammals and birds with a scaling exponent of ~ 0.74 (From West (2014); Adapted from Brody (1945))

In the previous discussion on scaling of metabolic rate to body mass, the metabolic rate used always concerns the standard metabolic rate (McNab, 1997; Hudson et al., 2013). However, these metabolic rates are originating from animals that are kept under standardized laboratory conditions (see above). When considering free-ranging mammals, e.g. free-living carnivores, the use of standard metabolic rates is less desirable since one would want to use a direct estimate of energy consumption in nature. The use of field metabolic rates (FMR) has made such an estimation possible and is typically assessed through the doubly labelled water technique (Speakman, 1997). Field metabolic rates are suggested to be more ecologically relevant (Hudson et al., 2013). The FMR of e.g. African wild dogs is known to be 5.2 x the standard metabolic rate during its normal activities (Gorman et al., 1998). Field metabolic rates have been scaled to body size (e.g. Nagy et al. (1999)). However, in general for mammals, the FMR scales to $M^{0.73}$ which is close to exponent 3/4 typically found for standard metabolic rates. For the mammalian order of Carnivora FMR scales to $M^{0.87}$ and the dietary group of carnivorous mammals scales to $M^{0.85}$. The latter is significantly different from and has a higher slope than other dietary groups such as insectivores and herbivores. However, this difference in scaling between dietary groups might be confounded

by taxonomic affiliation since these differences disappear within taxonomic affiliations (Nagy et al., 1999).

Food intake or ingestion is perceived to be directly related to energetic requirements or metabolic rate (see above, balanced growth equation) (Peters (1983); herbivores: Demment and Soest (1985); Illius and Gordon (1992)), which in turn, as explained before, scales to $M^{3/4}$. Empirical data sets indeed confirm that food intake scales similarly, also for carnivores. In 12 carnivorous species e.g., the absolute dry matter intake per day scaled to $M^{0.72}$ (Bourlière, 1975). The energy intake of 120 zoo animals, including ursids, viverrids, mustelids, felids, canids, procyonids scaled to $M^{0.75}$ (Evans and Miller, 1968). Farlow (1976) similarly reported energy intake to scale to $M^{0.70}$ for 100 carnivorous mammals.

2.2.2 *Gut capacity, food intake and retention times scaled to body size: herbivore insights*

Three major digestive variables, i.e. gut capacity, food intake and retention time and their interplay, are considered important digestive efficiency determinators as shown and extensively studied in herbivore species (Clauss et al., 2007b; Clauss et al., 2013). If gut capacity would be fixed, than an increase in food intake would lead to shorter retention times. If retention time would be fixed, than an increase in food intake would lead to an increase in gut capacity. The difference in allometric scaling to body mass between all three variables appeals to clarify species diversification and niche separation along a certain body mass range in mammalian herbivores, e.g. "How can herbivores of larger body size sustain themselves on lower quality diets?" (Müller et al., 2013). As gut capacity is known to scale almost isometrically to $M^{1.0}$ (Parra, 1978; Demment and Soest, 1985) and food intake to $M^{0.75}$ (cf supra), it implies that these different scalings result in a larger gut fill per unit food intake for increasing M. The latter has led to deductions concerning mean retention time (MRT) and digestibility in several ecological studies: the larger gut fill per unit food intake with increasing M implies an elongation of the retention time with increasing body mass (Demment and Soest, 1985; Illius and Gordon, 1992). Therefore the MRT should scale to $M^{0.25}$ ($M^{1.0-0.75}$) in mammalian herbivores. Since MRT is positively related to digestive efficiency in herbivores (Foose, 1982; Udén and Van Soest, 1982; Clauss et al., 2007b) this would imply that larger herbivores are more efficient at digestion, hence can tolerate lower quality diets (Demment and Soest, 1985; Illius and Gordon, 1992). However,

empirical data have shown that the scaling MRT ~ $M^{0.25}$ does not exist (Clauss et al., 2007a; Müller et al., 2011; Müller et al., 2013). Moreover, several deficits in the previous reasoning were adressed by Clauss et al. (2013) concerning the forage quality, the relation between digestibility and retention times and the overall digestive efficiency. Without going into detail, the authors pointed out that the gut capacity scaling higher than requirements, might allow larger herbivores to subsist on lower quality diets by just ingesting disproportionately more of them. As for the explanation why larger herbivores ingest low quality diets, this would result from ecological scenarios rather than physiological ones (Clauss et al., 2013; Müller et al., 2013).

Empirical datasets on gut capacity, food intake, retention times and additionally digestibility in carnivores have not been combined and analyzed so far. Considering the allometric relation with body size of each parameter might further establish regularities in order to explain species diversification in terms of physiology.

2.3 Body size versus animal ecology: the case of predator-prey interactions

Food webs are essential elements of ecosystems. This interconnection of food chains is established by consumer prey interactions that link different species in every food web (Estes, 1996). Consumer-prey interactions from a carnivore point of view are of a topdown-control, i.e. high level consumers such as carnivores depress the trophic level on which they feed (their prey) with an indirect increase in the next lower trophic level (Hunter and Price, 1992; Estes, 1996). Terrestrial carnivores are accordingly fundamental shapers of ecosystems and community structures through predation and intraguild interactions (Terborgh, 1992; McLaren and Peterson, 1994; Palomares and Caro, 1999; Ritchie and Johnson, 2009). Predator-prey interactions are considered indispensible when studying carnivores in terrestrial ecosystems (Cohen et al., 1993; Cohen, 1994; Woodward et al., 2005) and can be approached by the construction of empirical relations between predator size to the size of prey predated on (Rosenzweig, 1966; Paine, 1976; Woodward et al., 2005). Carnivore body size appears to be a driving factor in the choice for a specific prey size (Peters, 1983; Carbone et al., 1999; Carbone et al., 2014; Gervasi et al., 2014). Several allometric scalings have been established based on different empirical datasets in the form prey size = a predator size^b (Vézina, 1985; Carbone et al., 2014). These relationships are not,

however, concentrated on terrestrial mammalian carnivores solely but exceed the carnivore order level with datasets including predators within amphibians, snakes, birds and mammals (Vézina, 1985) and terrestrial mammals (Carbone et al., 2014). Scaling exponents obtained from general predator datasets are typically close to 1 (e.g. b = 1.05 for terrestrial mammals (Carbone et al., 2014)) (Fig. 4). However, Vézina (1985) obtained a scaling exponent of 1.18 for carnivorous feeders (including amphibians, snakes, birds and mammals) which exceeds a slope of 1. Overall, a slope close to 1 implies that prey size almost isometrically increases with predator size. A slope exceeding 1, as is the case for carnivorous feeders, implies that large predators take relatively larger prey compared to small predators. This is an effect that e.g. Carbone et al. (1999) observed, but rather than interpreting it as an ever-increasing ratio of prey:predator size, these authors introduced a cutoff at about 20 kg, below which carnivores typically consume prey much smaller than themselves, and above which carnivores take prey of a similar size as themselves. Large carnivores (> 20 kg) are more specialised in hunting and feeding on large vertebrate prey, i.e. prey of about or larger than their own bodymass whereas small carnivores (< 20 kg) tend to specialize in prey with a lower mass than their own body weight (including vertebrate and invertebrate prey) (Carbone et al., 1999; Carbone et al., 2007).

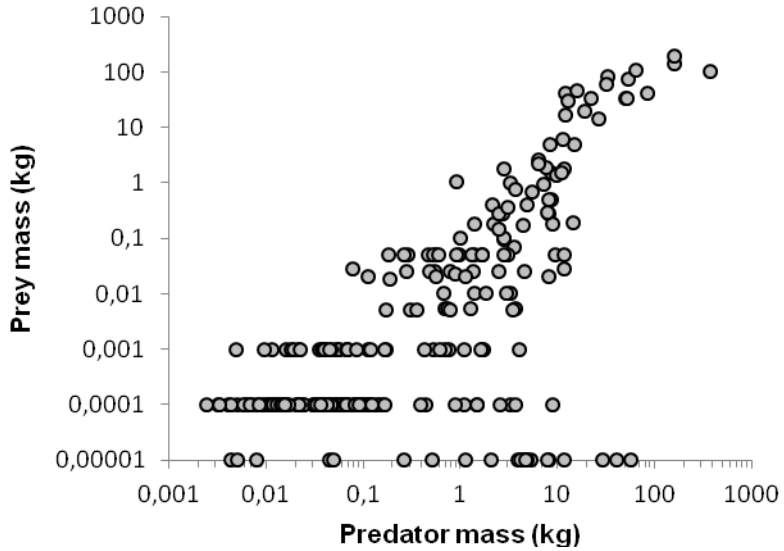

Fig. 4 Predator-prey size relationship for terrestrial mammals The scaling exponent of the linear relationship is 1.05 (Adapted from Carbone et al. (2014))

The underlying driver of prey size differences can be sought in mass related energetic requirements: small carnivores can subsist on a small vertebrate/invertebrate diet because of their lower absolute energetic requirements. However, these diets seem to be unsustainable for larger carnivores. A mass of ca. 20 kg is the maximum that can be sustained on small prey (Carbone et al., 1999), most likely due to a lacking availability of sufficiently dense stacks of small prey that could meet the high absolute requirements of large carnivores. Therefore, two functional groups within carnivores can be distinguished (small vs large carnivores), both representing distinct groups with own ecological and possibly physiological characteristics.

2.4 How body size could link carnivore feeding ecology with digestive physiology

The choice for a specific prey size is strongly determined by the body size and concomitantly by the energetic requirements of the carnivore (see 2.3) (Carbone et al., 1999), and the availability of a sufficient amount of available prey packages. In the study of predator-prey interactions, other fundamental elements related to body size such as the frequency at which carnivores consume prey (x kills/predator/unit time) (Holling, 1959; Vucetich et al., 2011) may yield important information on carnivore ecology. Apart from a scarce amount of field kill frequency data available in literature, efforts have been made to estimate predator kill frequency based on prey size and energetic requirements (Peters, 1983; Vézina, 1985). In order to do so, simply stated, data on predator daily food intake (or ingestion rate) are divided by the average prey size of the predator. Typically, these kill frequency estimates predict a decrease in kill frequency with (i) predator body size and (ii) prey size for a range of mammalian and avian carnivores with kill frequency (prey/day) = $28.8\ M^{-0.427}$ (Vézina, 1985). Larger carnivores are suggested to have less hunting obligations than do smaller carnivores. The latter shows how ecological features such as kill frequency might find their origins in physiological traits of the animal (i.e. body size hence energetic requirements) and point out the interplay between animal physiology and ecology.

The approaches used in the kill frequency modeling of Peters (1983) and Vézina (1985), however, do not take into account an important consequence from the predator-prey-size relationship: predators taking prey whose mass exceeds their intake capacity (i.e. larger predators) can feed selectively on their prey and will consume highly digestible body parts such as muscles and organs

(Hornocker, 1967; Bowland and Bowland, 1991; Stahler et al., 2006; Gidna et al., 2014; Bosch et al., 2015), while predators that kill comparatively small prey will consume their prey entirely (Mills, 1996; Bothma and Coertze, 2004; Anwar et al., 2011). Indeed, in literature it has been reported that large body-sized carnivores (focussed on vertebrate feeders), often sustain themselves on a 'feast-and-famine' regime associated with hunting on large prey. Large, highly digestible meals are alternated with periods of famine. A feast and famine regime may apply to short-term changes (i.e. having a hunting and eating day followed by a fasting and digestion day) and long-term changes such as extended periods of low prey availability (i.e. famine) alternating with periods of prey abundance (i.e. feast) (Bosch et al., 2015). This in contrast to small body-sized carnivores with their small prey, who adopt a more frequent feeding pattern with a regular food intake and a complete utilization of prey (e.g. the wildcat (Bradshaw, 2006)). The implications that prey size has on the maintained feeding strategy of a predator might be an overlooked principle when emperically looking at the scaling relationship of body size with ecological and physiological characteristics and their interplay, and requires further attention.

The differences in carnivore feeding strategies have been suggested to explain physiological differences observed between carnivore species (Bosch et al., 2015). The feeding strategy of obligate carnivores that have to hunt frequently during the day with a constant food intake (e.g. wildcat), might have enabled them to lose certain enzymatic pathways that facilitate synthethizing essential nutrients from endogenous stores during evolution (MacDonald et al., 1984; Morris, 2002). Similarly, other species with frequent-prey intake have been reported with adaptations in enzyme activity (i.e. mustelids, *de novo* synthesis of arginine; see section 1.2.2) (Leoschke and Elvehjem, 1959; Deshmukh and Shope, 1983) although lions (i.e. large carnivores with a typically feast and famine lifestyle) are not able to synthesise arachidonic acid from linoleic acid (Rivers et al., 1976). On the other hand, carnivores that can gorge themselves and hence adopt a 'feast-and-famine-regime' such as the wolf might have benefitted from maintaining such enzymatic pathways to cover essential nutrient production during days without prey intake (Kreeger, 2003; Bosch et al., 2015). The protein sparing capacity e.g., as seen in wolves, has also been described for other carnivores that have to cope with periods of famine (e.g. polar bear (*Ursus maritimus*) (Derocher et al., 1990)).

Next to metabolic adaptations to carnivore feeding strategies, the discrepancy in feeding strategies might also be reflected in morphometric characteristics such as gastric extension. Large prey enables predators to gorge themselves, i.e. ingest large quantities of prey which has been observed for several large mammalian carnivores (see above). Similarly, the choice for a specific feeding strategy might have implications for carnivore gut retention times when considering the close interplay between gut capacity, retention times and food intake (see 2.2.2). Given the assumption that a certain feeding strategy has implications for the nature of the digesta and the frequency at which the carnivore consumes prey (i.e. frequent feeding: complete prey ingestion at high frequency vs feast-and-famine: high quantity of highly digestible prey material at low frequency), this might lead to differences reflected in the gut retention time. Empirically studying retention times in relation to carnivore body size might further unravel adaptations to carnivore feeding strategy.

Gastrointestinal passage or retention time has been studied in carnivores in captivity and domestic carnivores (Bruce et al., 1999; Wyse et al., 2003; Boillat et al., 2010a; Elfström et al., 2013). Studying gastrointestinal transit in wild carnivores in captivity is useful when studying dietary composition based on faecal analysis in the wild, where incorporating gut retention time facilitates combining feeding habits with spatio-temporal behaviour (Elfström et al., 2013). An extensive amount of literature covers gastrointestinal passage in domestic carnivores, with the majority of studies concerning the domestic dog (C. familiaris) (e.g. Wyse et al., 2001; Rolfe et al., 2002). Such transit studies are often performed in order to establish reference standars for healthy individuals which should improve the diagnosis of gastrointestinal transit disorders (Bruce et al., 1999; Washabau, 2003; Wyse et al., 2003; Boillat et al., 2010a; Boillat et al., 2010b). Typically, gastric residence time or gastric emptying time is considered an important part of gastrointestinal passage and has been studied in dogs for species specific purposes (Boillat et al., 2010a), or as an animal model for human gastric motility in physiological and pharmaceutical studies (Wyse et al., 2003).

Currently, several methods are available to assess total digesta transit time in animals and even the transit through the different compartments of the gastrointestinal tract (e.g. gastric emptying time). The most simple way to assess total gut retention time is the application of a particle marker (be it powder or beads) to the diet, where the pattern of faecal marker concentrations makes it possible

to assess overall gut retention times (Thielemans et al., 1978). This method has been used predominantly in herbivore species (Steuer et al., 2010) but also in carnivore species (Tsuji et al., 2015). Given the need to study passage through different gut compartiments, several techniques have been developed and/or used in domestic carnivores such as diagnostic imaging techniques (e.g. radioscintigraphy), electric resistance techniques and tracer techniques (including gastric tracers, plasma tracers and breath tracers) (Wyse et al., 2003). Compiling data on the relation body size vs retention time might therefore not be as straightforward as is the case in herbivores where most retention time studies are restricted to a single method (see above). In carnivores, methodologies are widespread.

However, apart from these methodology differences, one major constraint in the emperical study of body size and retention time, from an evolutionary perspective, is the dramatic shift in 'diet type'. Not only did domestic carnivores switch from a high protein, low carbohydrate to a high protein, high carbohydrate diet (Plantinga et al., 2011; Bosch et al., 2015), a switch from a high level of physical structure present in whole prey (presence of hairs, bones, tendons, feathers, i.e. animal fibre; Depauw et al., 2013) to a less structured pelleted or canned diet should not be neglected. Evidently, current transit studies are mostly performed with commercial diets (kibble and canned diets) (Itoh et al., 1986; Peachey et al., 2000; Wyse et al., 2003; Boillat et al., 2010b). The physical structure of a diet can affect transit parameters, e.g. plant fibre particle size and dietary particle size can affect transit in herbivores, birds and humans (Vincent et al., 1995; Ferguson and Harris, 1997; Carré, 2000). Although the dietary physical structure effect has not been studied in carnivores, it might be that whole prey acts differently than the average commercial petfood diet and that different transit even occurs between different body parts present in whole prey. Hence, it is important to unravel how gastrointestinal passage is affected by whole prey feeding if one wants to study evolutionary adaptations to carnivore feeding strategies.

Carnivore body size might therefore be a determining driver of feeding strategies in the wild to which carnivores might have physiologically adapted. Digestive physiology diversity and the corresponding feeding strategy in the wild has extensively been studied in herbivorous mammals (Clauss et al., 2007a; Clauss et al., 2013; Müller et al., 2013) but is rather new in carnivore digestive physiology. The latter would offer more insight in digestive physiology of carnivores and lessons from the wild might be a key part in the management of carnivores in captivity and

domestic carnivores. The use of domestic carnivores as a model for wild carnivores or vice versa should not impose major drawbacks (see section 3.).

3. Domestic carnivores: the preservation of evolutionary adaptations

The most extensively studied domestic carnivores are the domestic dog and cat. Dogs were domesticated ca. 14.000 years ago and diverged from their wild ancestor, the wolf (*C.lupus*), when man shifted from a hunter-gatherer to a sedentary lifestyle (Vilà et al., 1999; Bradshaw, 2006; Galibert et al., 2011; Axelsson et al., 2013; Frantz et al., 2013). Cats were domesticated approximately 9000-10.000 years ago and diverged from a least five subspecies of the wildcat (*F. silvestris*), similarly as the dog, during the transition of man from hunter-gatherer to an agricultural lifestyle (Driscoll et al., 2007). The domestic dogs' and cats' genome seems to be strongly preserved compared to their wild ancestors (O'Brien and Yuhki, 1999; Murphy et al., 2000). Although breeding practices have led to a great morphological diversity among dogs and cats (Driscoll et al., 2009; Driscoll and Macdonald, 2010), this will not have affected certain physiological and metabolic traits in certain breeds since the breed-specific morphological traits are dominated by simple genetics (Lipinski et al., 2008; Boyko et al., 2010). The ability to synthesise essential nutrients and slow down the protein catabolism in wolves, e.g., is still present in domestic dogs (Legrand-Defretin, 1994; Kreeger, 2003; Bosch et al., 2015). Additionally, it would be very unlikely that dogs evolved certain physiological and metabolic traits that strongly differ from wolves (Meyer and Stadtfeld, 1980). However, dogs compared to wolves have evolved certain genetic mutations associated with starch digestion and glucose uptake which adapted them to the starch-rich diets commonly fed during domestication (Axelsson et al., 2013).

Current knowledge on the digestive physiology of the wild ancestors stems mostly from studies in the domestic descendants. Knowledge on the wolf's digestive physiology e.g. has been largely deducted from studies of the domestic dog (Peterson and Ciucci, 2003). Although one has to be careful with drawing parallels between wild ancestors and domestic descendants because of dramatic shifts in diets between the wild and domestic species (see above; Axelsson et al. (2013)), studying the digestive physiology in domestic descendants might expose evolutionary adaptations also present in wild counterparts. Challenging domestic species with natural-like diets (i.e. whole prey diets) has not been common practice so far. The digestion and metabolism of domestic cats, e.g, has been studied on raw meat diets (commercially available) and whole prey in order to model

digestive physiology of captive exotic felids (Vester et al., 2010a; Kerr et al., 2013; Kerr et al., 2014b). Similar studies might be of interest to expose any digestive physiological features on (preferably) whole prey diets. Additionally, unconventional diets such as whole prey feeding and raw meat feeding (e.g. bone and raw food (BARF)) are gaining more and more popularity in domestic carnivores (Schlesinger and Joffe, 2011; Freeman et al., 2013). Research concerning whole prey feeding is therefore imposing to unravel possible advantages and disadvantages where a knowledge on the whole prey associated digestive physiology is crucial.

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Scientific aims

The digestive physiology of terrestrial carnivorous mammals is characterised by a notable diversity among species. The occurrence of peculiar physiological and metabolic traits might find their origin in body size driven feeding strategies in the wild (Bosch et al., 2015). It has been well established that carnivore body size is a determining factor in the choice for a specific prey size with a switch from small to large prey feeding at a body mass treshold of ca. 20 kg (Carbone et al., 1999). Carnivore body size and the associated average prey size could further dictate a carnivore's feeding strategy. Reports in the literature describe large carnivores hunting prey larger than or similar to their own mass, typically ingesting large amounts of highly digestible food alternated with periods of famine (feast-and-famine adherents) (e.g. the wolf (Stahler et al., 2006; Bosch et al., 2015)); and, small carnivores that tend to specialize in prey with a lower mass than their own body weight that will typically ingest small, frequent meals in a non-selective way (e.g. wildcat (Bradshaw, 2006)). As such, it seems that carnivore body size drives a whole feeding strategy. However, the functional existence of both feeding strategies and their relation to carnivore body size has not been studied for a broad carnivore size spectrum (i.e. vertebrate-prey feeders) and could offer more insight in species diversification. Given the apparent difference in food intake, kill frequency and dietary composition between both feeding strategies, a difference in gut retention time can be expected. However, since gut retention can be affected by the physical structure of the diet (Ferguson and Harris, 1997; Carré, 2000) and since the majority of gastrointestinal passage studies in domestic carnivores and carnivores in captivity are conducted on traditional kibble diets or processed meats (Wyse et al., 2003; Boillat et al., 2010), gut retention time should be studied on whole prey diets (presence of physical structure) as a first step for future empirical relations of gut retention time and carnivore body size. Whole prey is characterised by more heterogeneity and structure and might affect gastrointestinal passage in ways that hitherto have been left unstudied.

In general, this dissertation aims to elucidate how carnivore feeding strategies have co-evolved with carnivore digestive physiology: Does carnivore body size drive the choice for the 'frequent-feeding' strategy and 'feast-and-famine' strategy? How does digestive processing (focussed on gastrointestinal transit) occur on a whole prey diet?

First, the feature kill frequency, considered an important part of a feeding strategy, will be modelled and scaled to carnivore body size. Kill frequency modelling will

account for several carnivore as well as prey characteristics: carnivore size, prey size, pack size, energetic requirements of carnivores, energy content in prey, gut capacity and selective feeding. Carnivores will be labelled feast-and-famine or frequent-feeding adherent based on the relationship prey size and gut capacity. The focus will be on vertebrate-prey feeding species given the different foraging strategies maintained by insectivorous and omnivorous species. The scaling of kill frequency with carnivore body size for both feeding strategies will render new information on the body size driven theory.

The second part of this dissertation aims to study passage through the carnivore gastrointestinal tract on a whole prey diet (varied in structure). The domestic dog (*Canis familiaris*) will be studied as a carnivore species in order to unravel all components of gastrointestinal passage (gastric emptying time, small bowel transit time, colonic transit time and total transit time) and faecal characteristics (consistency and fermentation profiles) on whole prey diets.

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Research chapters

1. Predator-prey size ratios determine kill frequency and carcass surplus production in terrestrial carnivorous mammals

Adapted from

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1.1 Abstract

Carnivore kill frequency is a fundamental part of predator-prey interactions which are important ecosystem shapers. Current field kill frequency data are lacking and existing models are insufficiently adapted to carnivore functional groups. We developed a kill frequency model accounting for carnivore mass, prey mass, carnivore specific maintenance energy requirements and metabolisable energy in prey, hunting pack size, selective feeding and carnivore gut capacity. Two main carnivore functional groups, small prey-feeders vs large prey-feeders, were established based on the relationship gut capacity (C) and pack corrected prey mass (iMprey); both groups have a linear scaling of the predator-prey size relationship, and although the majority of small prey-feeders is below, and of large prey-feeders above a body mass of 10-20 kg, both occur across the whole body size spectrum. Generally, kill frequency models predict an overall negative relationship between predator size and kill frequency, which we confirmed for large prey-feeders. However for small prey-feeders, this negative relationship was absent. When comparing carnivore prey requirements to estimated stomach capacity, small carnivores may have to eat to their full capacity repeatedly per day, requiring fast digestion and gut clearance. Large carnivores do not necessarily have to consume until they reach their maximal gastric capacity per day, or do not need to eat every day, which in turn reduces kill frequencies or drives other ecological processes such as scavenging, kleptoparasitism, and selective (incomplete) carcass consumption. The large prey-feeding strategy therefore appears particularly attractive for large carnivores, which can thus reduce activities related to hunting.

1.2 Introduction

Terrestrial carnivores are important drivers of the top-down control of ecosystems and the shaping of community structure, through both predation and intraguild interactions (Terborgh, 1992; McLaren and Peterson, 1994; Ritchie and Johnson, 2009). Predator-prey relationships are considered fundamental for studying terrestrial and marine ecosystems (Cohen et al., 1993; Heithaus, 2001; Carbone et al., 2014). Relating predator size to prey size is a commonly used approach to describe community interactions and feeding relationships at an interspecific level (Rosenzweig, 1966; Holling et al., 1976; Paine, 1976; Carbone et al., 1999). It is known that carnivore body size drives the choice for a specific prey size (Peters, 1983; Carbone et al., 1999; Carbone et al., 2014): a switch from small to large prey feeding occurs at a body mass threshold of about 20 kg (Carbone et al., 1999; Carbone et al., 2007). Small carnivores (<20kg) tend to specialize on very small prey (vertebrates and a considerable amount of invertebrates) whereas large carnivores (>20kg) will opt for large vertebrates equal to or exceeding their own mass (Carbone et al., 2007). Other important elements in predator-prey interactions, which are also related to body size, include the frequency at which carnivores consume prey (kills/predator/time) (Holling, 1959; Vézina, 1985; Vucetich et al., 2011), carnivore energetics (Carbone et al., 1999; Pawar et al., 2012; Carbone et al., 2014) and community structure (Nilsen et al., 2009; Vucetich et al., 2011). However, literature on field kill frequency data is rather scarce and almost exclusively available for large carnivores because of the labour intensive field methods and the fact that small prey items are mostly consumed entirely and therefore missed by field methods. Some papers report kill frequencies for small carnivores (e.g. van Aarde (1980); feral cat) but base the kill frequency on annual caloric requirement estimations rather than on direct observations. Others only consider prey-specific kill frequencies (e.g. the number of moose killed by wolves) and do not consider all prey species hunted by the carnivore (e.g. Zimmermann et al. (2015)).

Apart from field kill frequency data, efforts have been made to estimate carnivore kill frequency for a broad carnivore range based on carnivore prey size and energetic requirements (Peters, 1983; Vézina, 1985). Estimates show a decrease in kill frequency with (i) increasing carnivore body size and (ii) increasing prey size, which implies that smaller carnivores have more hunting obligations than do larger carnivores. For example, small cats need to kill multiple times per day (Bradshaw, 2006). Considering kill frequencies alongside prey size, prey energy content and energy requirements shows that smaller carnivores need to invest a significant portion of their day hunting (Jeschke, 2007). Larger carnivores can afford

to be 'lazy' since they can produce prey surplus on top of their energetic maintenance requirements.

The approaches used in previous modeling, however, do not explicitly account for a very important consequence of the predator-prey-mass relationship within vertebrate-prey feeders: predators taking prey whose mass exceeds their intake capacity can feed selectively on their prey, using only parts of increased energy density (Hornocker, 1967; Bowland and Bowland, 1991; Stahler et al., 2006; Gidna et al., 2014; Bosch et al., 2015), whereas predators that kill comparatively small prey will consume their prey entirely (Mills, 1996; Bothma and Coertze, 2004; Anwar et al., 2011). On the other hand, predators might not be able to fully consume their comparatively large prey, due to the limitation of their own intake capacity and the problem of preventing kleptoparasitism (scavenging) by other predators over an extended period of time (Carbone et al., 1997). Not considering predators separately whose prey does or does not exceed intake capacity may lead, for example, to estimates of kill frequencies for a 4 kg cat of 0.8 (Vézina, 1985) or 1.6 (Peters, 1983) times per day, rather than the 'multiple times' considered realistic for cats (Bradshaw, 2006). Therefore, we wanted to explore the relationship between kill frequency and carnivore body size and elucidate whether this varies across functional carnivore groups within the group of vertebrate-prey feeders (considering prey size in relation to intake capacity). In doing so, we develop a kill frequency model based on carnivore mass, the average of most common prey mass per carnivore, carnivore specific maintenance energy requirements and metabolisable energy in prey, hunting pack size, gut capacity, and the opportunity for selective feeding. Our working hypothesis was that if the mean prey mass available for the individual predator at a kill scaled lower than, or similar to, energy requirements, then no reduction in kill frequency and hunting obligation would occur with increasing predator mass; in contrast, if the prey mass available for the individual predator scaled higher than energy requirements, then a reduction in kill frequency would occur with increasing predator mass. We hypothesized that these results would be modified depending on the difference between prey mass and gut capacity.

1.3 Material and methods

1.3.1 *Data set*

A literature review was performed using Web of Knowledge, Pubmed and Google scholar to identify potentially eligible studies reporting feeding habits of wild carnivores. The literature search was conducted following Leenaars et al. (2012) by using two search terms, one based on the order of the Carnivora and the second on feeding habit associated factors. Aquatic carnivores or carnivores that depend on aquatic foraging strategies as well as carnivores of which the diet consists mainly (≥ 50 %) of vegetation and/or invertebrates were excluded from the database (based on the quantitative dataset on mammalian diets of Wilman et al. (2014)). The latter was done given the difference in foraging strategies between terrestrial vertebrate-prey feeders and insectivorous or omnivorous carnivores, and aquatic carnivores. Clearly, these foraging strategies differ in terms of search and feeding time (e.g. the difference in dispersal of terrestrial vertebrate and invertebrate prey; in the marine environment several small prey can be 'subdued' in the same hunting bout (swarms of fish) whereas this is not seen in terrestrial environments). The following data were extracted from each publication: carnivore species, study location, methods used for diet analysis, number of samples, carnivore sex, most frequent prey based on frequency or relative frequency of occurrence (FO= frequency of occurrence= identified prey items of a certain species/total number of scats (%); rFO= relative frequency of occurrence= identified prey items of a certain species/total number of prey items (%)), pack size (number of animals) (N_{pack}), kill frequency (1 kill/x days) (the 'real' kill frequency rKF) and maximal gut capacity (kg/carnivore/feeding event) (C). For the lion (*Panthera leo*), the spotted hyena (*Crocuta crocuta*), the tiger (*Panthera tigris*), the leopard (*Panthera pardus*), the cheetah (*Acinonyx jubatus*) and the African wild dog (*Lycaon pictus*), we included the reviews of Hayward and collaborators (Hayward and Kerley, 2005; Hayward, 2006; Hayward et al., 2006a; Hayward et al., 2006b; Hayward et al., 2006c; Hayward et al., 2012). Therefore, publications used in these reviews were excluded from the dataset. For pack size, maximal gut capacity and kill frequency data, additional literature searches were conducted.

Predator and prey mass (kg) (female and male average or range average) (M_{pred}, M_{prey}) were obtained from publications itself when authors were able to give typical carnivore and/or prey masses from the study area. Other carnivore and prey masses were mainly obtained from Nowak's Walker's Mammals of the world (Nowak, 1999), the panTHERIA database (Jones et

al., 2009) and the internet as a last reference. Other small dietary items were estimated at 0.001 kg for insects, 0.005 kg for aquatic invertebrates, 0.1 kg for small unidentified rodents and birds (Carbone et al., 1999). If studies expressed the most frequent prey as a group, class or order (e.g. small mammals, rodents), the average mass of that group (when given by publication) or all species included in that group, class or order was taken as mass of the most frequent prey. Whenever studies reported juveniles of a prey species as being the most frequent prey, juvenile prey mass given by the authors was used. If no juvenile prey mass was available, juvenile mass found in Nowak's Walker's mammals (Nowak, 1999) were used, or 10 % of the maternal prey mass was taken as representative of juvenile prey mass (Blueweiss et al., 2010). If the most frequent prey was confirmed to be carrion, the data point was omitted. If, for a certain carnivore species, the study gave the most frequent prey per location and/or per season/year/period, then prey was inserted per location and/or per season/year/period unless a total of all localities and/or periods was given. Whenever it occured that the frequency of occurrence of prey species A was lower than the frequency of occurrence of prey order B and prey order B had no species specification, then the frequency of occurrence of the prey order of species A was used to compare with the frequency of occurrence of prey order B. Whenever a study had two most frequent prey species that showed identical frequencies of occurrence, both were included in the database. If the study did not report frequency (FO) or relative frequency (rFO) of occurrence to point out the most frequent prey of a predator (e.g. indexes, consumed biomass,% of dry matter of scats) and/or FO and rFO could not be calculated from present measures (e.g. from consumed biomass), the study was excluded. Whenever the study itself mentioned that too few scats were analysed to determine the diet of a certain carnivore species, that part of the study was excluded. Per carnivore species, 10 publications (or less if no more than 10 were available), focussing on reviews, were added to the database.

Observed kill frequency data (rKF) were corrected for the pack size of the carnivore species, obtained from the publication itself (i.e., dividing the reported frequency with pack size). Kill frequencies that apply only to a specific prey species (e.g., the number of moose killed by wolves, irrespective of other prey taken in the same time period) were not taken into account since these estimates did not consider all prey species hunted by the carnivore (Kroshko et al., 2016). Per carnivore species, the average of most common prey mass (M_{prey}), the average N_{Pack} and the average rKF were calculated.

1.3.2 *Kill frequency modelling*

A theoretical kill frequency (KF) model was developed based on M_{pred}, M_{prey}, carnivore specific maintenance energy requirements (Q_{pred}) and metabolisable energy in prey (E_{prey}). For each species, KF is calculated as Q_{pred} / E_{prey}.

Based on the scaling relationships of

$M_{prey} \sim M_{pred}^{p}$

$Q_{pred} \sim M_{pred}^{q}$

and the assumption that the energy content of prey is directly proportional to prey mass, we would expect

$KF \sim M_{pred}^{(q-p)}$

However, given the occurrence of pack hunting, and our considerations about feeding selectivity and gut capacity, several modifications to this simple concept need to be applied. Under the assumption that pack size scales with predator mass

$N_{pack} \sim M_{pred}^{n}$

the amount of prey available for the individual predator (iM_{prey}) scales to

$iM_{prey} \sim M_{pred}^{(p-n)}$

and therefore

$KF \sim M_{pred}^{(q-p+n)}$

Note that a scaling of pack size with body mass may not be expected, but data for individual species must be corrected for pack size nevertheless.

The relationship of $M_{prey} \sim M_{pred}^{p}$ needs to be established for several groups of predators, in relation to their gut capacity C. We divided predators into those where $iM_{prey} < 1\%$ of C (i.e., predators mainly preying on insects), those where 1% of $C < iM_{prey} < C$ (or 'small prey pedators'), and those where $C < iM_{prey}$ (or 'large prey predators' who cannot consume their average prey in one meal). The metabolisable energy of whole prey was estimated at 5348 kJ/kg fresh weight calculated from data given by Plantinga et al. (2011), and prey items < 5 kg were considered to be completely edible whereas prey items of > 5 kg were considered 95% edible. For selective feeding, prey was considered to be consumed as 70% (Mills, 1990; Stander, 1992; Caro, 1994), at a metabolisable energy content of 8048 kJ/kg fresh weight (the

average value given by Bosch et al. (2015) taking into account the selective feeding of wolves (*Canis lupus*)). Large prey predators were assumed to only consume the equivalent of their gut capacity C per day. For this group, KF estimates were either based on a single-day feeding on their prey (in a selective mode, i.e. eating the amount of C at 8048 kJ/kg) or a complete consumption of their prey (in a non-selective mode, i.e. with 95% consumption at 5348 kJ/kg), to outline theoretical minimum and maximum kill frequencies.

Following Nagy et al. (1999), we parameterize the relationship of $Q_{pred} = b\ M_{pred}^{q}$ as $Q_{pred} = 791kJ\ M_{pred}^{0.85}\ d^{-1}$.

Evaluations of scaling relationships were performed using linear regressions for log-transformed data in ordinary least squares (OLS) in R using the package nlme (Pinheiro et al., 2011). To account for the phylogenetic structure of the dataset, data were linked to a phylogenetic tree (Fritz et al., 2009), and also analysed in phylogenetic generalized least squares (PGLS) with the phylogenetic signal λ estimated by maximum likelihood, using the package caper (Orme et al., 2010). Extrapolation to other species (for C) was based on OLS scaling, because PGLS scalings are based on phylogenies that do not include the species to which the extrapolation is to be applied. Because we considered the polar bear (*Ursus maritimus*) as an extreme example of a predator that might switch between comparatively small prey (fish) and large prey (seals), we excluded this species from scaling analyses, and used it as an example for the range of kill frequencies available to large carnivores with the option of such a large prey range. For comparison, the KF models of Peters (1983) and Vézina (1985) were included in the graphs representing our KF.

1.4 Results

1.4.1 *Carnivore characteristics*

A total of 456 studies, 513 prey size datapoints, 182 pack size datapoints, 56 kill frequency datapoints and 22 gastric capacity datapoints were incorporated in the database. Seventy eight carnivore species were included in the prey size database. Pack size data could only be obtained for 75 carnivore species. Real kill frequency data were obtained for 11 carnivore species. Carnivore weight ranged from 0.1375 to 387.5 kg. Data on the maximal gastric capacity (C) were available for 9 species ranging from 0.19 kg to 150.0 kg; these data were used to determine the allometric function [with 95% CI] of $C = 0.09\ [0.06;0.14]\ M_{pred}^{1.19\ [1.07;1.30]}$, which was used to calculate the C for all carnivore species. A compilation of the carnivore families and species included in the dataset for KF modelling can be found in **Appendix 1.**

Of the 75 carnivore species for which pack data were available, 12 species were pack hunters and 63 species were solitary hunters. Pack size scaled nominally to 1 [1;1] $M_{pred}^{0.14\ [0.05;0.24]}$. Of the 12 pack hunting species, 7 species had a $C < iM_{prey}$, i.e. the pack could not consume the whole prey animal in one day. Five pack hunting species had larger C than iM_{prey}, i.e. were supposedly sharing prey that each individual could have eaten more of - the yellow throated marten (*Martes flavigula*, 2 pack members), the golden/asian jackal (*Canis aureus*, 2.5 pack members), red wolf (*Canis rufus*, 2.4 pack members), Ethiopian wolf or simien jackal (*Canis simensis*, 5.7 pack members), and bush dog (*Speothos venaticus*, 11 pack members). Of these, only the bush dog had a higher M_{prey} than C (i.e. the pack was killing prey that would have been too large to be consumed by an individual member). Therefore, the bush dog appeared as an outlier in the graph displaying the M_{pred}-M_{prey} relationship (Fig. 1a), but not in the graph linking M_{pred} to iM_{prey} (Fig. 1b).

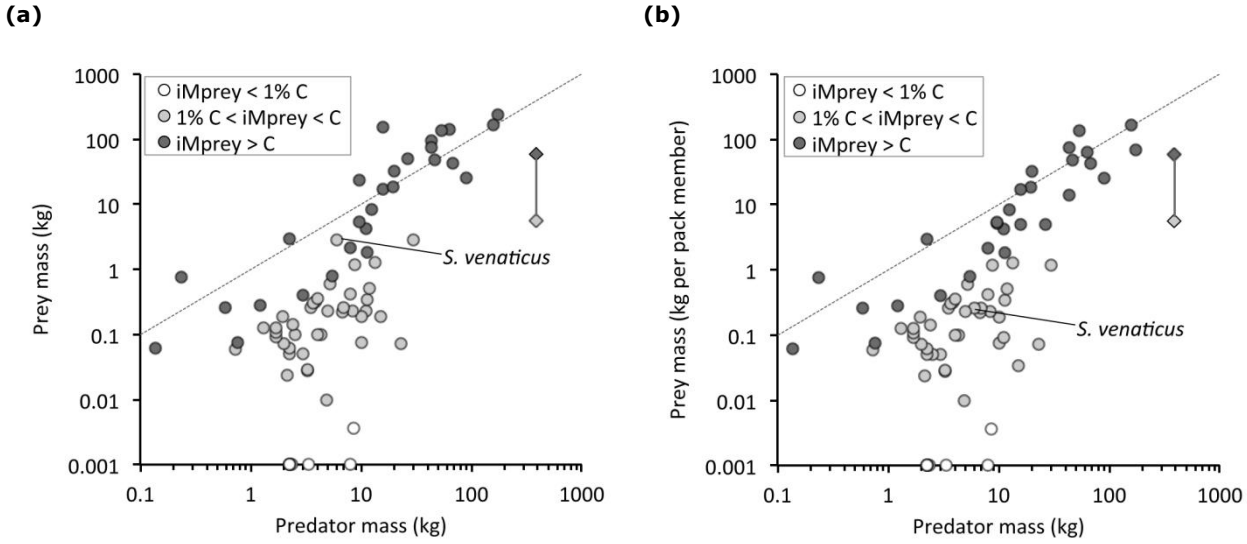

Fig. 1 Relationship between individual predator species mass and (a) average prey mass or (b) average prey mass divided by the number of pack members (iM_{prey}) The dotted line represents y=x (prey mass = predator mass). Predators are groups according to their iM_{prey} relative to their stomach capacity C. The linked diamonds indicate the two ecotypes of the polar bear (*Ursus maritimus*, see text). Note that due to its comparatively large prey and large pack size, the bushdog (*Speothos venaticus*) is an outlier in (a) but not in (b). For statistics, see Table 1.

Real kill frequency data were found for 11 species weighing between 11.2 kg and 175.5 kg. Using species averages, the real kill frequency scaled to 1.11 [0.20;6.18] $M_{pred}^{-0.48\ [-0.91;-0.04]}$, and did not show a phylogenetic signal (λ not significantly different from 0).

1.4.2 *Predator-prey mass scaling*

Across all carnivore species, prey mass scaled to predator mass with a scaling exponent larger than 1.0, also exceeding linearity in the 95% confidence interval (Table 1). In contrast, when considering carnivore groups individually based on relative prey size, linear scaling was included in the 95% confidence interval of both small and large prey predators and hence also overlapped between these groups (Table 1). However, there was a large difference in the scaling factor (intercept), which was 0.05 for small prey predators and 0.5 for large prey predators, with no overlap in the 95% confidence intervals (Table 1).

Table 1 Scaling relationships of prey mass (M_{prey}) or prey mass available for the individual predator (iM_{prey}) with predator mass (M_{pred}) according to $a\ M_{pred}^{b}$ in different datasets (depending on the relationship between stomach capacity C and iM_{prey}) using ordinary least squares (OLS) or phylogenetic generalized least squares (PGLS)

Dependent variable	Dataset	n	Statistic	λ	*a* (95%CI)	*p*	*b* (95%CI)	*p*
M_{prey}	whole	74	OLS	(0)	0.03 (0.01;0.07)	<0.001	1.56 (1.17;1.96)	<0.001
			PGLS	0.220*	0.02 (0.00;0.08)	<0.001	1.74 (1.30;2.18)	<0.001
	$1\%C < iM_{prey} < C$	40	OLS	(0)	0.05 (0.02;0.10)	<0.001	0.81 (0.40;1.22)	<0.001
			PGLS	0*	-	-	-	-
	$iM_{prey} > C$	27	OLS	(0)	0.52 (0.27;0.98)	0.056	1.19 (0.98;1.41)	<0.001
			PGLS	0.189*	0.53 (0.24;1.17)	0.129	1.23 (0.99;1.47)	<0.001
iM_{prey}	whole	74	OLS	(0)	0.03 (0.01;0.06)	<0.001	1.42 (1.04;1.80)	<0.001
			PGLS	0.321**	0.02 (0.00;0.09)	<0.001	1.60 (1.18;2.02)	<0.001
	$1\%C < iM_{prey} < C$	40	OLS	(0)	0.06 (0.03;0.11)	<0.001	0.64 (0.24;1.04)	<0.001
			PGLS	0*	-	-	-	-
	$iM_{prey} > C$	27	OLS	(0)	0.45 (0.26;0.78)	0.009	1.08 (0.90;1.27)	<0.001
			PGLS	0*	-	-	-	-

* λ significantly different from 1; ** λ significantly different from 0 and 1

Table 2 Scaling relationships of kill frequency with predator mass (M_{pred}) according to $a\ M_{pred}^{b}$ in different datasets (depending on the relationship between stomach capacity C and prey mass available to the individual predator iM_{prey}) using ordinary least squares (OLS) or phylogenetic generalized least squares (PGLS); for large prey predators, the kill frequency assuming a single meal per prey (i.e., constrained by C) or a complete consumption of the prey (i.e. over several days, assuming an absence of scavenging/kleptoparasitism) are indicated

Dataset	n	Statistic	λ	*a* (95%CI)	*p*	*b* (95%CI)	*p*
whole	74	OLS	(0)	6.95 (3.31;14.56)	<0.001	-0.53 (-0.86;-0.20)	0.002
		PGLS	0.270**	9.12 (2.71;30.70)	0.001	-0.66 (-1.01;-0.30)	0.001
$1\%C < iM_{prey} < C$	40	OLS	(0)	2.64 (1.30;5.36)	0.010	0.20 (-0.20;0.61)	0.332
		PGLS	0*	-	-	-	-
$iM_{prey} > C$	27	OLS	(0)	1.01 (0.96;1.06)	0.652	-0.33 (-0.34;-0.31)	<0.001
(single meal)		PGLS	0	-	-	-	-
$iM_{prey} > C$		OLS	(0)	0.33 (0.19;0.58)	0.001	-0.22 (-0.40;-0.04)	0.024
(complete consumption)		PGLS	0*	-	-	-	-

* λ significantly different from 1; ** λ significantly different from 0 and 1

1.4.3 *Kill frequency model outcomes*

The overall KF scaling in the complete dataset had a very steep scaling with an exponent of -0.66 (Table 2). However, when considering predator groups individually, small prey predators did not have a significant scaling of KF with body mass (Fig. 2), and had a mean of 7 ± 12 kills per day. In contrast, large prey predators had a significant negative scaling, which was shallower if complete consumption of prey was assumed (Table 2). The range of kill frequencies for large prey predators was between two kills per day and a kill every 29 days (Fig. 2).

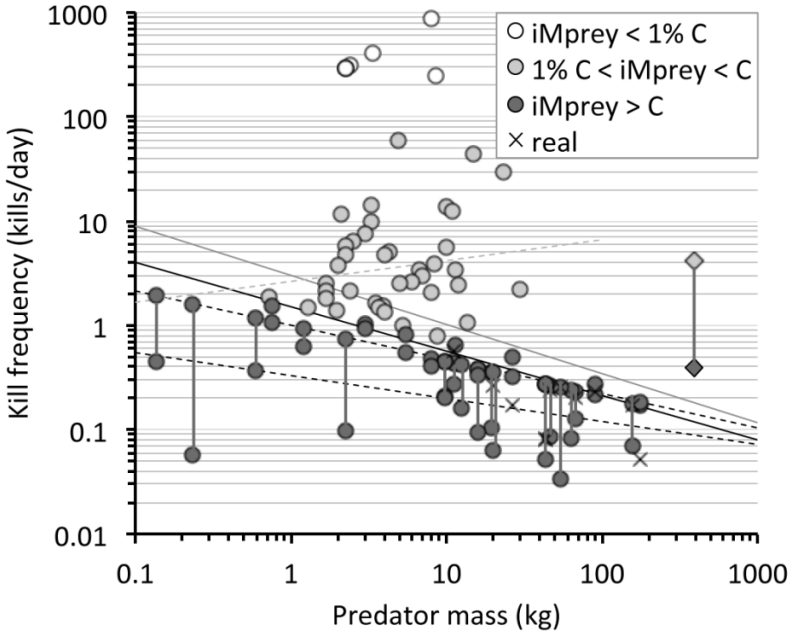

Fig. 2 Relationship between individual predator species mass and the kill frequency (days per killing event) necessary to meet energy demands for the average prey items. Predators are groups according to their iM_{prey} relative to their stomach capacity C The linked diamonds indicate the two ecotypes of the polar bear (*Ursus maritimus*, see text). For large prey predators, the two linked data points indicate the kill frequency assuming a single meal from the prey (upper points, constrained by stomach capacity), and the kill frequency assuming that the prey can be consumed completely (i.e., over the course of several days, without scavengers or kleptoparasites), with dark dotted lines indicating the respective regression lines. The bright dotted line indicates the (non-significant) regression line for small prey predators. Real kill frequencies reported in the literature (for sources, see Online Resource 1,2) are indicated as crosses. The light grey line represents the model by Peters (1983), the dark grey line the model by Vézina (1985). For statistics, see Table 2

The polar bear (*Ursus maritimus*) is an example of a species that, depending on its seasonal ecotype (i.e., ice bound, winter prey = seal; land bound, summer prey = mixed, e.g. geese and fish) (Russell, 1975; Dyck and Romberg, 2007; Gormezano and Rockwell, 2013), might have

a very low kill frequency or be a very selective feeder in winter, and be a non-selective feeder with a higher kill frequency in summer (Fig. 2).

When plotting the required amount of prey as well as the estimated stomach capacity against M_{pred} (Fig. 3), it appears that small predators have to eat more than their stomach capacity per day (i.e., must also have a gut clearance of less than a day), whereas large predators from approximately 4 kg upwards can, in theory, ingest more prey per day than required and thus might not need to hunt the same number of prey items on a daily basis; from a body mass of approximately 30 kg upwards, large predators could theoretically eat to their full stomach capacity (or hunt prey of sufficient size) only once every other day .

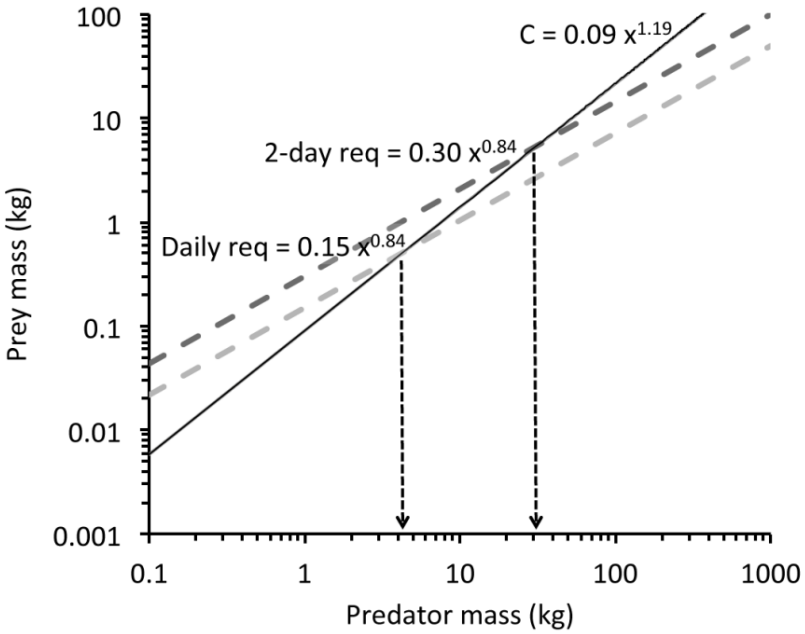

Fig. 3 Comparison of the scaling of stomach capacity C and the daily prey mass requirement with predator mass Theoretically, below 4 kg, predators have to ingest more than their stomach capacity (i.e., have a shorter stomach passage than one day), whereas above 4 kg, predators can afford to eat less than their stomach capacity per day, and above 30 kg, predators can afford to only hunt/eat every second day

1.5 Discussion

The results of our study demonstrate that depending on the prey size selected by terrestrial mammalian predators, these predators may vary distinctively in their kill frequencies and hence their daily activity budgets and hunting behaviours. While for the group of predators that, on average, subdue prey that exceeds their instantaneous intake capacity a decrease in kill frequency with body size is a model output, similar to the general findings of Peters (1983) and Vézina (1985), such a relationship with body size is not evident in predators focussed on prey of a size that is smaller than their instantaneous intake capacity. Notably, our dataset showed that whether or not predators focus on prey larger or smaller than their own instantaneous intake capacity is not necessarily a function of body size, because some smaller predators also apparently pursue such a large prey-feeding strategy. However, there was the well-described general pattern that below 10-20 kg of body mass, more predators were 'small prey-feeders' that hunt for prey well below their instantaneous intake capacity, whereas above 10-20 kg, predators mainly go for comparatively larger prey (Carbone et al., 1999). One of the major implications for the ecological impact of predators is that they can be classified into those that only hunt what they will necessarily consume for themselves, and those that potentially (but not necessarily) create a surplus of prey (for themselves, or the wider community of scavengers or kleptoparasites) because they cannot completely consume their prey instantaneously.

As evident from Fig. 2, our model yields results that differ in relevant ways from those of previous estimates of carnivore kill frequencies. In particular, the most similar previous models by Peters (1983) and Vézina (1985) seem to mainly reflect the behaviour of 'large prey-feeders' and not those of 'small prey-feeders' that live on small vertebrates. The data bases of these authors include (i) both avian and mammalian carnivores, (ii) an estimation of the scaling of energy requirements derived from caloric intake data of mainly captive animals from (Farlow, 1976), and (iii) use a range of prey sizes according to their proportion in overall prey intake. By contrast, our models are based on the main average prey size, on energy requirements estimated on the basis of a regression of known field metabolic rates against body mass (Nagy et al., 1999), as well as on information on pack size, and a certain accounting for selective feeding in 'large prey-feeders'. Given the broadness of either approach, we do not claim our model to be superior, but consider its main relevance in unveiling a dichotomy in potential ecological impact between predator categories. A quick check of the consistency of our approach is that even if we know that there is no significant

scaling of pack size with body mass, the scaling exponents should theoretically correspond to $KF \sim M_{pred}^{(q-p+n)}$. In the whole dataset, q = 0.85 due to our use of the regression of Nagy et al. (1999), and in OLS p = 1.56 (Table 1) and n = 0.14. Therefore, we would expect KF to scale, in OLS, to 0.85-1.56+0.14 = -0.57, which corresponds closely to the resulting exponent of -0.53 in Table 2. Deviations from the expected exponent are due to the additional data scatter introduced by adjustment factors for carcass use (complete vs. proportions) and prey energy density. When compared to the scaling of real kill frequencies, which were available from the literature exclusively for large prey-feeders, the large confidence interval of its scaling exponent ($M_{pred}^{-0.48\ [-0.91;-0.04]}$) included the scaling for large prey-feeders in our model ($M_{pred}^{-0.33\ [-0.34;-0.31]}$ for the single meal and $M_{pred}^{-0.22\ [-0.40;-0.04]}$ for the complete consumption strategy). Differences in the real scaling exponent to model estimates are most likely due to either specific characteristics of the populations under observation, or an under-representation of smaller prey in observational studies (due to unintended bias in behavioural observations towards large prey) (Sunquist, 1981).

Possibly the most important factor that was implicit, but not explicitly stated in the previous models is gut capacity C. In the Peters (1983) and Vézina (1985) models, daily food intake and energy requirement was set at the scaling derived from the unpublished food intake data (given as kcal/d) collected by Farlow (1976). Therefore, in those models, energy requirements and food intake could not differ. By contrast, our model used different estimates for intake capacity (as stomach capacity C) and energy requirement that had different scaling exponents (Fig. 3). Conceptually, this approach accounts for the experimental observation that food intake increased asymptotically with larger M_{prey} (Wachter et al., 2012; Chakrabarti et al., 2016). As long as the mass of an individual prey item (M_{prey}) is smaller than C, M_{prey} is the main driver of kill frequency in our dataset. As soon as M_{prey} is larger than C, however, C becomes one factor constraining KF. If we assume the different scaling between requirements and gut capacity (Fig. 3) to be true for carnivores, this also means that smaller carnivores would have to eat, within the same day, repeatedly (to their gut fill), either from the same large carcass or several small prey items, to meet their energy needs. Correspondingly, assuming intake at C, the model yields two kills - or, in this case, eating events - for a small-bodied large prey-feeder (a mustelid). This can only be achieved if gut clearance (or digesta passage or retention time) is < 24 hours. At published retention times of 1.96 to 11.75 h in mustelids (e.g. Japanese marten *Mustela melampus*; Tsuji et al. (2015)), this condition is apparently given.

The situation assumed in our carnivore model, of a discrepancy in the scaling of gut capacity on the one, and requirements on the other hand, resembles a concept in herbivores where gut capacity also scales higher than requirements, thus potentially allowing larger herbivores to subsist on lower quality-diets by just ingesting disproportionately more of them (Clauss et al., 2013; Müller et al., 2013). In carnivores, the discrepancy between gut capacity and requirements does not buffer against a lower quality-diet, but allows larger intervals between hunting (and even eating) events. Evidently, this potential can only be used if these larger carnivores pursue a strategy of large prey-feeding. Although larger carnivores could in theory also meet their energy demands by ingesting many small prey items (such as the Ethiopian wolf *C. simensis* in our dataset), the combination of their relatively large gut capacity and the ecological availability of large prey allows them to reduce hunting efforts (Carbone et al., 2007), feed selectively on the large prey they acquire, and become 'full and lazy' (Jeschke, 2007).

The other constraint that determines the lower range of kill frequencies is how well a predator can defend or hide a carcass against kleptoparasites/scavengers, so that it can eat from it again on subsequent days. The way in which scavenging on surplus killed carcass might affect kill frequency was adressed and modelled by Andrén et al. (2011) for the Eurasian lynx *(Lynx lynx)* and wolverine *(Gulo gulo)*. Both species prey upon reindeer *(Rangifer tarandus)* with the wolverine (facultative scavenger) being more prone to scavenging lynx (obligate predator) kills. Their model outcomes showed that wolverine kill frequency decreased when lynx-wolverine ratio increased from 1 to 2, i.e. lynx provided more scavenging opportunities. Another phenomenon that can be driven by surplus killing is kleptoparasitism, i.e. competing predators that try to steal a carcass from other predators. Kleptoparasitism is described for several species, e.g. the brown bear (*Ursus arctos*) that can displace the lynx from one third of its kills; or the African wild dog *(L. pictus)* and the cheetah *(A. jubatus)*, both losing their prey to lions and hyaenas (Kruuk, 1972; Schaller, 1972; Gorman et al., 1998). To protect their prey surplus and overcome kleptoparasitism and the production of waste, carnivores might opt for alternative strategies for carcass use. Subordinate predators such as cheetah or wild dogs may choose the time of day or the geographic location of their hunts so that contact with superior predators is minimized (Stander, 1990; Mills and Gorman, 1997). A social option to defend carcasses is pack hunting and feeding, which enables carnivores to defend their carcasses more succesfully and consume the prey faster and more completely (Lamprecht, 1978; Lamprecht, 1981). Elbroch (2017) mentions that conspecific animals (individuals of the same

species) can aggregate at kill sites although the species are solitary hunters (e.g. pumas, leopards and tigers). Another way of coping with carcass surplus is to make the carcass inaccessible to scavengers or klepotparasites, as the leopard *(P. pardus)* often does by moving prey into trees, caves, large burrows or dense vegetation (Sunquist and Sunquist, 2002; Balme et al., 2017). Food-caching has been described in wolves, bears (eg. *U. maritimus*), hyaenids, felids (eg. bobcats and tigers), and mustelids (Harrington, 1981; Sunquist, 1981; Phillips et al., 1990; Vander Wall, 1990). Thus, some carnivores can feed for several days on the same carcass. Alternatively, if resources and conditions allow, they may choose to not even consume a carcass, but only its most nutritious parts, and rather hunt new prey than consume the less digestible portions (Stirling and McEwan, 1975; Gende et al., 2001).

Mid-to-small-sized carnivores show a large variety in kill frequency with our model approach (Fig. 2). Most mid-sized carnivores are not limited by their maximal gastric capacity. The main cause lies in their choice of small prey. This may also be due to the low availability of large prey in their habitat (cf. Ethiopian wolf) or a lack of sociality in these species that otherwise would help to overcome large prey (Lamprecht, 1978). In comparison, larger predators may be limited in their ability to prey on smaller prey species due to the challenge of obtaining a sufficient amount of these comparatively small packages. Because small prey items cannot be filtered out of a terrestrial environment but must be comprehended individually, larger animals are limited in the prey size they can pursue, in contrast to marine predators that can filter small prey items out of their environment (Carbone et al., 2014). The fact that large-bodied carnivores produce surplus means they provide excess food provision for themselves for several days, and/or create opportunities for scavenging (Wilmers et al., 2003). Especially with respect to non-mammalian scavengers, mammalian predators therefore are important facilitators in their ecosystems that render large bodied-prey parts available for other species.

It has been suggested that differences in the foraging mode are the potential causes of physiological differences observed between carnivore species (Bosch et al., 2015). Obligate carnivores that have to hunt repeatedly in the course of a day, and therefore have a constant intake of animal matter, might have been in a position where they could afford, during evolution, to lose certain enzymatic pathways that facilitate synthethizing essential nutrients from endogenous stores (Morris, 2002). On the other hand, carnivores that can gorge themselves and hence adopt a 'feast-and-famine-regime' might have benefitted from maintaining such enzymatic pathways to cover essential nutrient production during days

without prey intake. Given this reasoning, we might expect similarities in enzymatic pathway properties between the cat and other carnivorous species with a frequent intra-diurnal prey intake, such as mustelids. For example, MacDonald et al. (1984) reviewed evidence that mustelids share some peculiarities with domestic cat enzymatic equipment, e.g. in arginine metabolism. However, the same authors also report similarities between cats and larger, feast-and-famine type of felids, such as lions, for example with respect to the inability to synthesize arachidonic acid. Possibly, phylogenetic peculiarities led to different enzymatic characteristics of different carnivore species irrespective of convergence in their prey size and feast-and-famine foraging behaviour.

Findings on kill frequencies may have several implications for the feeding of captive (domestic and non-domestic) carnivores. The wildcat *(Felis silvestris)* for example is a solitary hunter that mainly focuses on prey with a lower body mass (e.g. rodents, birds) which makes it necessary to make several kills per day (MacDonald et al., 1984; Bradshaw, 2006). Nowadays, domestic cats or wild cats kept in zoos are often offered single meals per day, which may contribute to the increasing problem of obesity in at least in domestic cats (Laflamme, 2006; Bissot et al., 2010; Deng et al., 2013). Increasing feeding frequency is a recently adopted strategy (amongst others) to manage body weight in domestic cats (Deng et al., 2013). On the other hand, non-domestic carnivores in captivity such as the lion often suffer from problems related to dietary over-supply (i.e. obesity, inactivity and stereotypy). In a study where lions were gradually adapted from a conventional feeding program to a random gorge feed/fasting day program, it was observed that food digestibility and body weight improved (Altman et al., 2005). Such a feeding schedule, which includes the provision of more prey than can be immediately consumed, would also help facilitate the expression of natural behaviour related to caching or carcass guarding. Elucidating feeding strategies in the wild such as the relationships between predator size, prey size and kill frequency is therefore a key part in the management of carnivores *ex situ*.

In conclusion, our model outcomes seem to coincide with our predictions that as predator mass increases (i.e., at larger body sizes), the intake capacity exceeds the energetic requirements, leading to a reduction in kill frequency if the typical prey individual exceeds the predator's stomach capacity. Kill frequency outcomes for small prey-feeders were more variable, mostly not limited by gut capacity, and therefore not resulting in a kill frequency reduction. Thus, a functional dichotomy seems to exist in carnivores, but to what extent phylogenetic, physiologic or ecologic factors determine whether a carnivore is a 'small prey-

feeder' or a 'large prey-feeder', apart from a broad but not exclusive pattern of a body size threshold at about 10-20 kg, remains to be fully explored. In particular, this dichotomy might also occur within species or even individuals over time. The functional dichotomy may well occur within species where different individuals are specialized on diffent prey (Codron et al., 2016), within individuals over ontogeny (Elbroch et al., 2017), or in individuals between hunting events (Lumetsberger et al., 2017). Observations deviating from the general pattern, such as a population of wild cats living on rabbits rather than small rodens (Malo et al., 2004), or a population of wild dogs living mainly on very small ungulates (Woodroffe et al., 2007), indicate that the underlying cause for the dichotomy must be sought in ecological circumstances rather than fixed physiological and behavioural adaptations.

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2. How does dietary particle size affect canine gastrointestinal transit: A comparison of dietary markers

Adapted from

De Cuyper A[1], Hesta M[1], Tibosch S[2], Wanke C[2], Clauss M[3] and Janssens GPJ[1] (2017) How does dietary particle size affect carnivore gastrointestinal transit: A dog model. Journal of Animal Physiology and Animal Nutrition (In press)

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2.1 Abstract

The effect of dietary particle size on gastrointestinal transit in carnivores has not been studied and might offer more insight in their digestive physiology. This study evaluated the effect of two dietary particle sizes (fine = 7.8 mm vs coarse = 13 mm) of chuncked day old chicks on transit parameters in dogs. Six beagle dogs were fed both dietary treatments in a cross-over design of 7 days with transit testing on the fifth day. Transit parameters were assessed using two markers, i.e. a wireless motility capsule (IntelliCap®) and titanium oxide (TiO_2). Dietary particle size did not affect gastric emptying time (GRT), small bowel transit time (SBTT), colonic transit time (CTT) and total transit time (aTTT) of the capsule ($P > 0.05$). There was no effect of dietary particle size on TiO_2 mean retention time (MRT) ($P > 0.05$). The time of last TiO_2 excretion (MaxRT) differed ($P = 0.013$) between diets, being later for the coarse diet. Both MRT ($R = 0.617$, $P = 0.032$) and MaxRT ($R = 0.814$; $P = 0.001$) were positively correlated to aTTT. The ratio MRT/aTTT tended towards a difference between diets ($P = 0.059$) with the coarse diet exceeding fine diet values. Results show that the difference between capsule measurements and TiO_2 is larger for the fine than the coarse diet suggesting that the capsule becomes more accurate when dietary particle size approaches marker size. Dietary particle size might have affected transit parameters but differences are too small to claim major physiological consequences.

2.2 Introduction

Texture is a complex dietary characteristic influenced by several factors such as hardness, viscosity and many others (Chen and Rosenthal, 2015). Particle size is one such factor that affects dietary texture and might influence digestive physiology. It is known that dietary particle size affects digesta passage in ruminants (Udén, 1988). In monogastric animals, particle size of plant-derived fibre in the diet can affect gastrointestinal transit times in humans, horses, rabbits, rats, pigs and poultry (Heller et al., 1980; Stanogias and Pearce, 1985; Gidenne et al., 1991; Vincent et al., 1995; Ferguson and Harris, 1997; Carré, 2000; Svihus et al., 2002; Van Weyenberg et al., 2006). However, little information exists on the effect of dietary particle size variation on transit times in carnivores. In dogs, the effect of varying texture - although not by particle size but by adding insoluble plant-derived fibre - on gastric emptying and/or total transit time has been studied to some extent. According to Burrows et al. (1982), the inclusion of cellulose in a canine diet decreases total transit time. By contrast, Pedreira et al. (2013) showed that the inclusion of 10 % insoluble fibre (sugarcane fibre) in a dog's diet delayed the gastric emptying and colonic filling time.

Elucidating how dietary particle size influences gastrointestinal passage rate in carnivores might offer more insight in carnivore digestive physiology. It is known - although not well substantiated - that extending gastric emptying time in dogs may help to influence satiety. Gastric fill and stomach extension followed by a subsequent slowing of gastric emptying (Weber et al., 2007) might be an important mechanism through which dogs get satiated. Pappas et al. (1989) found that gradual gastric distention caused gradual inhibition of food intake in a non-cholinergic way and that satiety was not influenced by the nutrient content of the food. Given that free-ranging carnivores can be expected to swallow larger chunks of their prey compared to domestic animals fed processed feeds, there might be a general difference in the level of satiety experienced between free-ranging and domesticated carnivores.

We want to elucidate how particle size, as a texture-influencing factor, affects gastric emptying time and total transit time in carnivores. For this aim, the dog was studied as a carnivore species. To remain true to the carnivore's natural diet, particle size was varied in a complete animal based diet (Plantinga et al., 2011; Bosch et al., 2015) rich in animal fibre (i.e. poorly digestible animal tissues ((glyco)protein-rich matter such as raw bones, tendons, cartilage, skin, hair or feathers)) (Depauw et al., 2013). Currently, several methods are available for assessing transit in dogs (Wyse et al., 2003) but not all are equally accurate, noninvasive and practical. Recently, wireless motility capsules that are administered orally

and measure pH and temperature throughout the gastrointestinal tract have been used successfully to assess passage rate in dogs (Boillat et al., 2010a). Therefore, transit was monitored using the IntelliCap® system (Medimetrics, Personalized drug delivery group, the Netherlands) together with the control marker titanium oxide (TiO_2).

2.3 Material and methods

2.3.1 *Animals and housing*

Six healthy adult laboratory beagle dogs (*Canis lupus familiaris* L.) (four females and two males) aged between two and seven years with a body weight between 9 and 14.1 kg and body condition score between 3 and 5 out of 9 were housed individually in neighbouring kennels at the Laboratory of Animal Nutrition of Ghent University (Merelbeke, Belgium). The number of experimental individuals (n = 6) was based on the study of Boillat et al. (2010a).

2.3.2 *Experimental design and diets*

Experimental procedures were approved by the Ethical Committee of the Faculty of Veterinary Medicine of Ghent University (EC2015/45) following the EU and Belgian Government-established norms and procedures. Dogs were fed two test diets in a cross-over design of seven days with passage rate testing on the fifth day of both periods. The test diets consisted exclusively of chunked day-old-chicks (Kiezebrink Putten B.V., Hoge Eng Oost, the Netherlands) and differed only in particle size. The fine diet had a particle size of 7.8 mm whereas the coarse diet had a particle size of 13 mm (KOLBE AW 130 meat mincer; die size fine diet = 7.8 mm; die size coarse diet = 13 mm) (Fig. 1). Because of the limited duration of the trial, it was chosen not to adjust the diet for eventual deviations from nutrient requirement guidelines (i.e. minerals and vitamines), in order to keep the intervention simple.

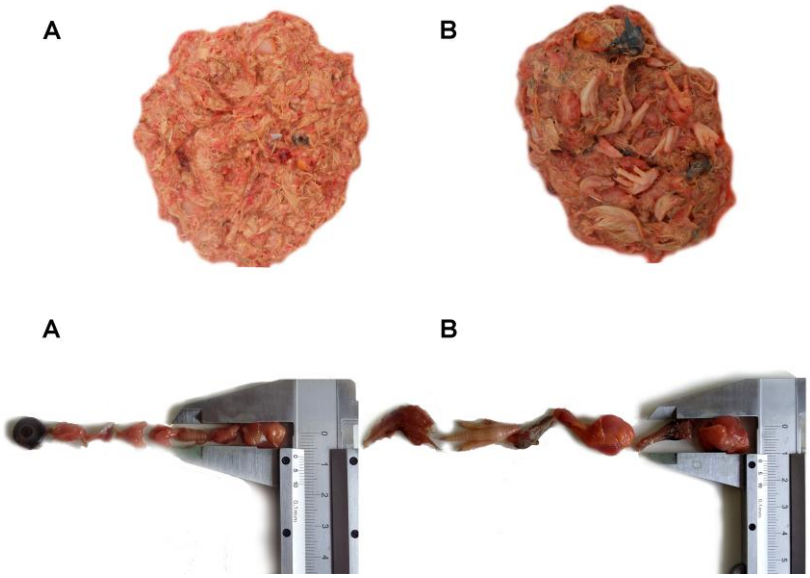

Fig. 1 Chunked day-old-chicks
Particle size 7.8 mm (A) and 13 mm (B); particles can be lower or exceed the average particle size within a diet which is visible in the depicted caliper measurements

The nutrient composition of the diet is shown in Table 1. Dry matter (DM) and ash contents were determined by drying to a constant weight at 100 °C and combustion at 550°C, respectively. Crude protein (6·25 x N) was analysed using the Kjeldahl method (ISO, 2005a) and crude fat was analysed according to the Soxhlet method (ISO, 1973). Total fibrous matter and insoluble fibre were analysed according to the method of Cools et al. (2015). This method is based on the in vitro digestive simulation of Boisen and Fernández (1995) and Hervera et al. (2007) and resembles the TDF analysis according to Prosky et al. (1985) with this difference that the fibre fraction obtained, includes not only the plant-derived carbohydrate fraction (TDF) but also animal fibre (protein-rich). Amino acids were analysed using the ISO 13903 method (2005b).

Table 1 Nutrient composition of chunked day old chicks

	Chuncked day old chicks
Nutrient composition, % of DM	
Dry matter	24.9
Crude protein	57.3
Crude fat	22.7-26.4[a]
Total fibrous matter	38.0
Insoluble fibre	26.2
Crude ash	7.1
Crude fibre	2.5
Amino acid composition, g/kg DM	
Cysteine	14.5
Taurine	5.6
Methionine	14.7
Aspartic acid	57.6
Threonine	28.5
Serine	37.9
Glutamic acid	83.7
Glycine	47.7
Alanine	38.9
Valine	34.7
Isoleucine	28.3
Leucine	50.5
Tyrosine	20.8
Phenylalanine	32.2
Histidine	16.3
Lysine	39.7
Arginine	39.0
Metabolisable energy, kJ/100g DM	1672[b]

DM, dry matter; [a] smallest value without hydrolysis, largest value with hydrolysis; [b] The ME is the average of the values calculated by Atwater factors (16.7 * Crude protein + 37.7 * crude fat + 16.7 * NfE and the alternative predictive equation of the NRC (2006) (with NfE (Nitrogen free extract; 100 - moisture% - Crude protein% - Crude fat% - Crude fibre% - Crude ash%)

Before the onset of the trial all dogs were fed with chunked day-old-chicks (13 mm) for three weeks. During the first week, the chunked chicks were gradually added to the usual kibble diet (0% to 100 % chunked chicks of MER). The following two weeks, dogs were meal fed exclusively with chunked day-old-chicks (13 mm) according to their maintenance energy requirements (based on NRC requirements (NRC, 2006) for adult laboratory dogs) to maintain constant bodyweight, which was assessed weekly. Five of the six dogs were willing to consume the diet from the beginning and consumed it within 5 minutes. One dog was more reluctant whereupon its meal was spread throughout the day. After the adaptation period, the cross-over trial was executed with dogs being meal fed every day according to their individual maintenance energy requirements. Each dog always received the same amount of food

throughout the cross-over experiment so there was no difference in food intake between dietary treatments. The mean food intake (as fed) was 907.1 g/day/dog (± 348.4 g). All dogs had ad libitum water access.

2.3.3 *Gastric emptying and gastrointestinal passage*

On the fifth day of every test period, gastric emptying and gastrointestinal transit was monitored by the IntelliCap® system (Medimetrics, Personalized drug delivery group, the Netherlands). The IntelliCap system consists of an electronic capsule that can be administered orally and measures pH and temperature throughout the gastrointestinal tract. This renders information on capsule location in the gastrointestinal tract. Capsule sizes were 11 mm diameter by 26.7 mm long (Zou et al., 2013). All dogs were offered their daily meal in the morning. A maximum of 30 minutes was allowed to ingest the meal. All dogs finished their meal within the first 5 minutes except for one, which finished in 30 minutes. Before administration, IntelliCap® capsules were assembled according to Medimetrics standard operating procedures. After every dog had finished its meal, the IntelliCap capsule was administered by deep throat deposition followed by a rinse (approximately 20 mL) of drinking water to assist swallowing. A single IntelliCap® portable unit (data receiver) was mounted at the front of each kennel. From administration until excretion of the capsule, pH and temperature were measured and reported every 60 seconds until deactivation of the capsule.

Administration and excretion of the capsule were determined by the temperature profile and render a good estimate for the total transit time (aTTT). Gastric emptying is characterized by a quick rise in pH. Gastric residence time (aGRT) was therefore defined as the time interval between capsule administration and the abrupt increase in pH profile, i.e. passage of the pylorus. After entering the small intestine there is a steady rise in pH followed by a pH plateau phase. Afterwards the pH suddenly drops by about 1.0 pH unit or more. This decrease indicates transit through the ileocolic valve. The time between the entry into the small bowel and the entry into the cecum was defined as the small bowel transit time (aSBTT). Finally, the colonic transit time (aCTT) was defined as the time between the cecum entry and the excretion of the capsule from the body (Fig. 2) (Zou et al., 2013). Additionally, relative (r) GRT, SBTT and CTT were calculated by dividing the absolute value by the aTTT.

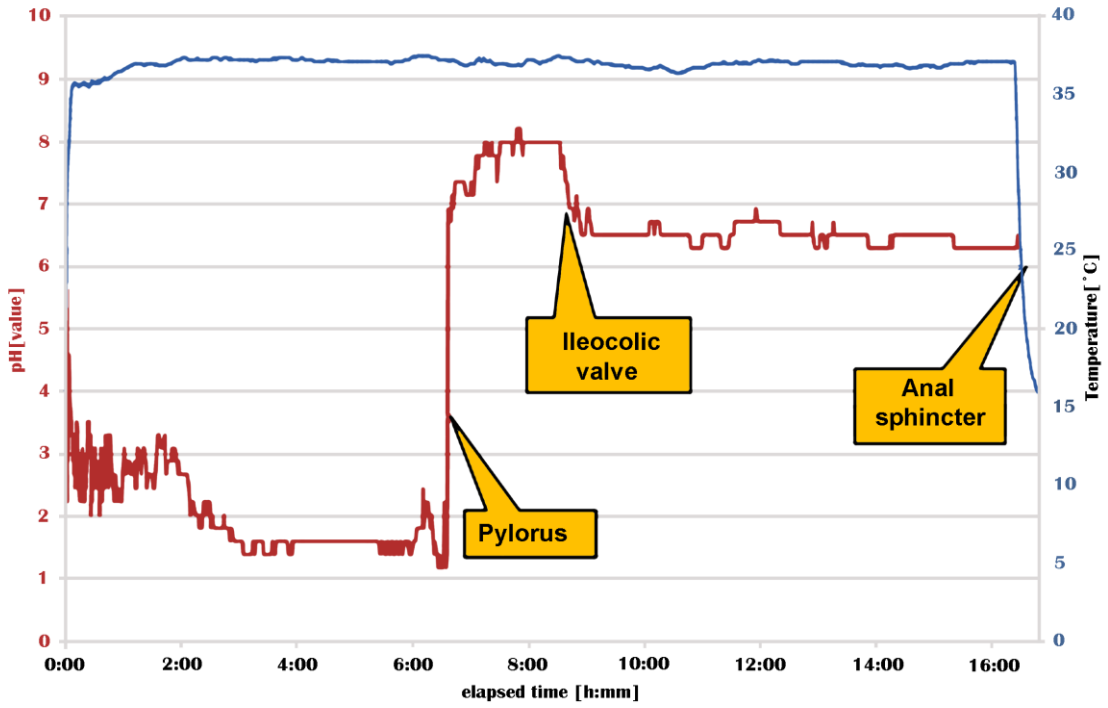

Fig. 2 pH and temperature profile of a beagle dog gastrointestinal tract

Additionally, on the same day as the capsule administration, both diets were enriched with 2 g/kg TiO_2 (VWR, International BVBA, Leuven, Belgium) as a control marker for total transit time. For each dog, the marker was mixed manually with the diet until a visually homogenous distribution of the marker was achieved. Faecal samples were collected constantly from one day before TiO_2 addition to the diet until two days after the TiO_2 addition. Faecal samples were always collected within 15 minutes after defecation. Faecal samples were weighed, dried at 60 °C and analysed for Ti following Myers et al. (2004) and the mean retention time was calculated. Mean retention time (MRT) of TiO_2, the best single measure of rate of passage through the gastro-intestinal tract, was calculated using the Thielemans method (Thielemans et al., 1978):

$$MRT\ (h) = \Sigma\, t_i\, C_i\, \Delta t_i / \Sigma\, C_i\, \Delta t_i$$

where C_i is the marker concentration in the interval indicated by time t_i (hours after marker administration) and Δt_i = the interval of the concerning sample:

$$\Delta t_i = ((t_{i+1} - t_i) + (t_i - t_{i-1})) / 2$$

Furthermore, the time of last marker excretion (MaxRT) (< 5% of the max. concentration) was registered for every dog and treatment. Additionally, the ratio MRT/aTTT and MaxRT/aTTT was calculated for both dietary treatments.

2.3.4 *Statistical analysis*

Statistical analyses were performed using Superior Performing Software Systems version 23 (SPSS, Chicago, IL, USA). A paired T-test was applied to compare the parameters aGRT, aSBTT, aCTT, aTTT, rGRT, rSBTT, rCTT, MRT, MRT/aTTT, MaxRT and MaxRT/aTTT for both diets. Additionally, a paired T-test was applied to total transit measurements within diets for both types of marker (MRT vs aTTT; MaxRT vs aTTT).

Pearson correlations were done between all capsule transit measures to test eg. if a longer aGRT is linked to a longer aCTT (aGRT vs aSBTT; aGRT vs aCTT; aGRT vs aTTT; aSBTT vs aCTT; aSBTT vs aTTT; aCTT vs aTTT; rGRT vs rSBTT; rGRT vs rCTT; rSBTT vs rCTT). Additionally, MRT and MaxRT were correlated with aTTT to compare the IntelliCap® method to the TiO_2 marker method.

2.4 Results

All dogs remained healthy throughout the study and consumed all provided food every day. The treatment with the IntelliCap system was safe and well tolerated by every dog. All capsules were excreted and recovered intact. On the fifth day of the first testing period when capsules were administered, one dog refused to eat the whole amount of food provided within the limited amount of time (356 g vs 808 g). Subsequently, on the fifth day of the second test period, this dog was offered the same diminished amount of food to be able to compare test periods. The pH and temperature profile per treatment and per dog can be found in **Appendix 2**.

Although aGRT, aCTT and aTTT were numerically lower on the coarse diet, there were no capsule transit time differences between diets ($P > 0.05$) (Table 2). The average total transit time of the capsule was 30.5 ± 10.6 h across diets. This aTTT consisted of constant proportions of 0.50 rGRT, 0.09 rSBTT and 0.42 rCTT that did not differ between diets. Both aGRT ($R = 0.825$; $P = 0.001$) and aCTT ($R = 0.913$; $P < 0.001$) but not aSBTT ($R = 0.344$; $P > 0.05$) were positively correlated with aTTT. As expected, rGRT was negatively correlated with rCTT ($R = -0.950$; $P < 0.001$), but neither one was correlated with rSBTT.

Table 2 Average transit parameters for two test diets (7.8 mm vs 13 mm)

	Fine diet (7.8 mm)		Coarse diet (13 mm)		
	Mean	SD	Mean	SD	P
Absolute capsule times, hours					
aGRT	15.4	6.6	13.7	3.8	0.36
aSBTT	2.6	0.75	2.4	0.34	0.60
aCTT	14.8	8.3	12.2	5.1	0.46
aTTT	32.8	13.5	28.2	7.5	0.35
Relative capsule times, % of aTTT					
rGRT	48.3	10.7	48.8	10.2	0.93
rSBTT	9.1	4.4	8.7	1.9	0.80
rCTT	42.5	12.3	42.5	10.2	0.99
Mean retention time, hours					
MRT	19.5	5.1	22.0	3.8	0.16
Maximum retention time,					
MaxRT	30.8	10.6	33.3	9.6	0.013
Ratio MRT vs aTTT					
MRT/aTTT	0.67	0.24	0.81	0.17	0.059
Ratio MaxRT vs aTTT					
MaxRT/aTTT	0.97	0.12	1.19	0.21	0.17

aGRT = absolute gastric residence time; aSBTT = absolute small bowel transit time; aCTT = absolute colonic transit time; aTTT = absolute total transit time; rGRT = relative gastric residence time; rSBTT = relative small bowel transit time; rCTT = relative colonic transit time; MRT = mean retention time; MaxRT = maximum retention time; n = 6 dogs

The TiO_2 recovery averaged at 81.2% (SD = 12.9) for the fine diet and 73.7% (SD = 8.2) for the coarse diet. The mean retention time (MRT) did not differ between dietary treatments ($P > 0.05$) (Table 2). The average MRT was 20.8 ± 4.5 h across diets. However, the timepoint of last marker excretion (MaxRT) differed significantly ($P = 0.013$) between diets, with the coarse diet exceeding the average value of the fine. Both MRT ($R = 0.617$, $P = 0.032$) and MaxRT ($R = 0.814$; $P = 0.001$) were positively correlated to the aTTT (Fig. 3). The slope of the respective regression equations was < 1 (0.136, 0.497) for the MRT-aTTT relationship, but included 1 in the confidence interval (0.368, 1.113) for the MaxRT-aTTT relationship. Within diets, MRT and aTTT differed (fine diet: $P = 0.026$; coarse diet: $P = 0.032$). The difference between MaxRT and aTTT was not significant within the fine diet ($P = 0.4$) but tended towards significance in the coarse diet ($P = 0.074$).

The difference between the diets in the ratio MRT/aTTT tended towards significance ($P = 0.059$). The ratio showed higher values (closer to 1) for the coarse diet compared to the fine diet meaning that the MRT lies closer to the aTTT for the coarse diet compared to the fine

diet. The MaxRT/aTTT ratio was 0.97 vs. 1.19 for the fine and coarse diet, respectively (P = 0.17), indicating that on the coarse diet, the capsule tended to be excreted sooner than the last titanium marker.

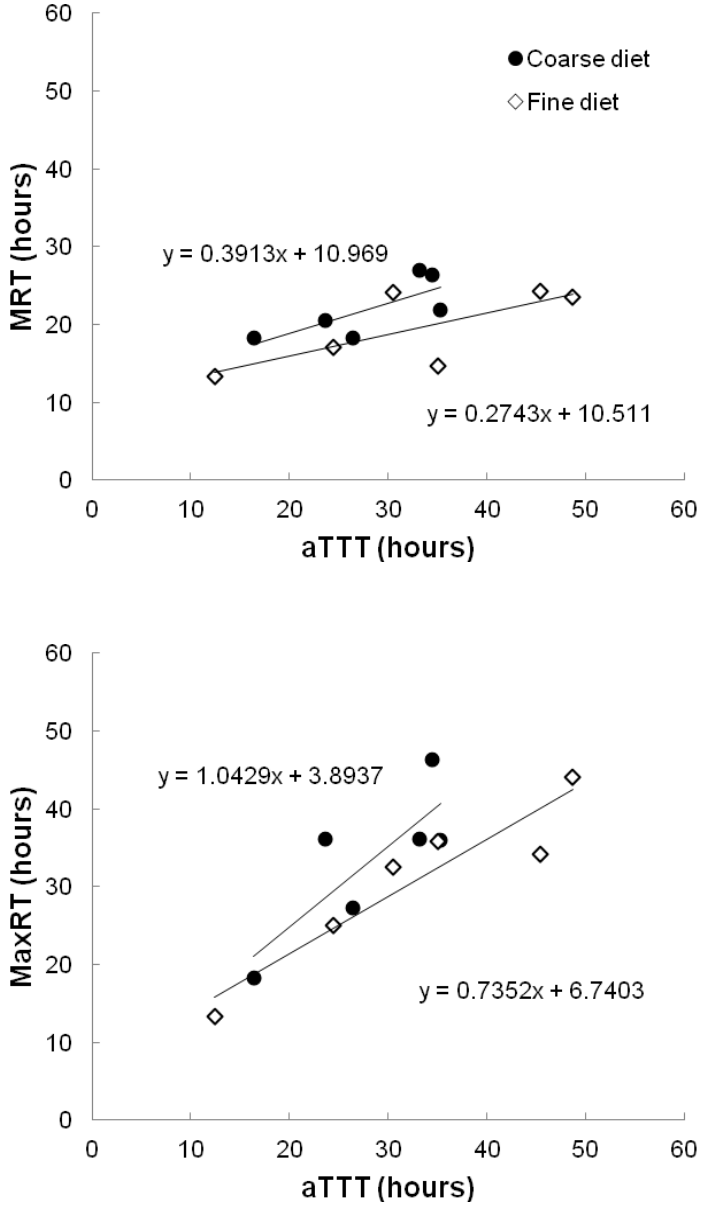

Fig. 3 Correlations between marker retention times and capsule total transit time MRT = Mean retention time (TiO_2); MaxRT = timepoint of last marker excretion or maximum retention time (TiO_2); aTTT = total transit time (capsule); n = 6 dogs

2.5 Discussion

Overall, dietary particle size did not affect gastric emptying time and did not affect other transit parameters with the exception of MaxRT; however, dietary particle size appeared to influence how the two marker systems (a powder applied to the diet, or a larger capsule) compared with each other. Therefore, although our data does not indicate major changes in transit parameters, it indicates that the difference in particle size between diet and marker can have a relevant effect on measurements.

2.5.1 *Effects of dietary particle size with constant marker size*

Gastric emptying

Our results show that dietary particle size does not seem to have an effect on gastric emptying time (aGRT). We would have expected the coarse diet to slow down the gastric emptying compared to the fine diet. In humans, Vincent et al. (1995) showed that coarse bran compared to fine bran slowed down gastric emptying, although this concerned fibre particle size and not complete dietary particle size as is the case in our study. In general, the effect of dietary particle size on gastrointestinal transit in carnivores has not been studied. Adding texture to a canine diet - not by increasing particle size but through the inclusion of insoluble fibre (which might unknowingly have lead to the addition of more coarse particles) - delayed gastric emptying (Pedreira et al., 2013). However, we could not observe this effect in this study where texture was varied through particle size variation. The main reason probably lies in the particle size difference between the two test diets which was only 5.2 mm and too small to provoke significant differences. Additionally, particle size variation within one diet might have undone the particle size difference between diets (Fig. 1). However, when considering studies in herbivores and birds, particle size differences of µm's or mm's have been shown to affect gut retention times (Ferguson and Harris, 1997; Carré, 2000). Given the absence of a frame of reference concerning dietary particle size in carnivores, a particle size difference of a mm difference range was hypothesized to provoke an effect on carnivore gut retention time. A 5.2 mm difference was the largest difference that could be obtained due to limitations of the food processing equipment. It might be that large particles from both diets were retained in the stomach until the interdigestive migratory myoelectric complex (IMMC) occured since they both exceeded 5 mm of particle size (see below). However, the latter threshold sizes concern non-food indigestible particles which differ from dietary particle kinetics. Dietary particles that are too large to pass the pylorus will be propulsed back into the stomach and will

be reduced in size through mechanical breakdown via repetitive muscular contractions until they are able to pass the pylorus (Wyse et al., 2003; Martinez and Papich, 2009). Additionally, due to the plasticity and form of food-particles, it is still possible that larger particles are 'moulded' through the pylorus and thereby react differently than indigestible solids (Carré, 2000). From this point of view, the fine diet (7.8 mm) still might have left the stomach earlier than the coarse diet (13 mm) although the aGRT was not affected by the diet type. However, any small difference in aGRT between the fine and coarse diet might have been missed mainly due to the fact that the capsule reacts as an indigestible solid with the aGRT reflecting the time at which the whole solid meal has left the stomach and not the 'average' food particle residence time.

Small bowel transit, colon transit and total transit

Neither of the other capsule-measured transit times (aSBTT, aCTT, aTTT, rSBTT and rCTT) nor the TiO_2-MRT were affected by dietary particle size. Only the MaxRT was affected by dietary particle size. The effect of particle size on small bowel transit has hardly been studied. Our results show similar SBTT values (2.5 h ± 0.56) as found by Boillat et al. (2010a; b) that also used a wireles motility capsule to study gastrointestinal transit times in dogs. The SBTT found in this study reflects the typical speed at which the IMMC propulses through the small bowel (Code and Marlett, 1975) and does not show any significant effect of particle size. As aSBTT (in contrast to aGRT and aCTT) was not correlated to aTTT, small intestinal transit appears to be particularly consistent, whereas transit through the other sections of the gastrointestinal tract are subject to more variation.

Additionally, no effect of particle size was seen on the aCTT. However, colonic transit times tend to show high intraindividual variability (Boillat et al., 2010a) which was also observed in our study and might be due to the control of defecation by the dog for reasons unrelated to digestive physiology. Therefore, it might be difficult to observe differences in CTT provoked by particle size. Total transit time (aTTT) was not affected by particle size as well which is something we would have expected based on other species. Van Weyenberg et al. (2006) e.g. reviewed passage rate in horses and its influencing factors. Whenever feed particle size is reduced, the mean retention time is increased, particularly in the colon. By adding more texture to horse diets by e.g. long hay, the passage rate is increased compared to smaller particles in e.g. pelleted diets. The authors do differentiate between total dietary particle size and fibre length. Reducing the fibre length can shorten the mean retention time in the gut although this can vary according to the fibre source. Similarly as for the aGRT, it might still

be that the particle size difference between diets in this study was too small to evoke any difference in aSBTT, aCTT and aTTT. However, the most plausible explanation for the aTTT is the size of the marker. In contrast to the aTTT, the MaxRT did experience a significant effect of diet type, i.e. particle size with the coarse diet having a longer MaxRT than the fine diet suggesting that the TiO_2-marker was more sensitive than the wireless motility capsule, and that the diet might have had some effect on digesta transit.

2.5.2 *Effects of marker size with constant diet*

Marker size seemed to influence transit measurements. Our results showed that within diets, TiO_2-MRT values and aTTT capsule values were significantly different with the aTTT exceeding the MRT value. The MaxRT did not significantly differ from aTTT within the fine diet but showed a tendency towards significance within the coarse diet. Clearly, the difference in marker size (i.e. powder vs large inert capsule) seemed to affect transit parameters, and this was probably mainly at the level of the stomach. In dogs, Itoh et al. (1986) reported that increasing the particle size of inert radio-opaque markers slowed down gastric emptying. Nelson et al. (2001) reported that increasing particle size (of barium impregnated spheres) increased the duration of the time needed to reach a certain percentage of gastric emptying but it also increased the interindividual variability in gastric emptying time. It is known for dogs that objects of different size - as in non-food accidentaly ingested - to differ in the time at which they leave stomach. Dressman (1986) reviewed and reported that particles of ≤ 1.6 mm leave the stomach sooner than the meal, and once particles exceed 2.4 mm, the particles are expelled later than the meal. Martinez and Papich (2009) state that particles $\leq$ 2-3 mm should be able to pass the canine pylorus immediately. Others state that the threshold lies at ≤ 5 mm diameter (Itoh et al., 1986; Wyse et al., 2003). Once exceeding the previously mentioned diameters, non-food particles are retained in the stomach until the interdigestive migratory myoelectric complex (IMMC) occurs, which propels large particles towards the duodenum (Itoh et al., 1986; Wyse et al., 2003). Once the stomach is passed, there does not seem to be an effect of marker size on the rest of the transit through the gastrointestinal tract. Also Bruce et al. (1999) did not see any difference between the CTTs in dogs of small inert radiopaque polyethylene spheres (1 mm) and large spheres (5 mm).

In our study, capsule sizes were 11 mm diameter by 26.7 mm long. Consequently, the capsule was not able to pass the pylorus and will have left the stomach with the IMMC for both diets. By contrast, the powder of the TiO_2-marker will have left the stomach earlier together with the food. Without subdivision in dietary treatments, the MRT (obtained by the TiO_2-marker)

however did positively correlate with the aTTT (capsule) (Fig. 3) although the aTTT was always higher than the MRT. The latter is logical since the MRT represents the mean retention time of the food in the gut (Thielemans et al., 1978) whereas the capsule aTTT represents the transit time of the last food that left the stomach (Martinez and Papich, 2009). Therefore, the MaxRT is a more comparable measure for aTTT, and indeed the MaxRT was more strongly correlated to the aTTT (R = 0.814 compared to R = 0.617) (Fig. 3). However, the important question might be how the marker size compares to the particle size of the diet fed with that particular marker.

2.5.3 *Difference in particle size between diet and marker*

The dietary difference for the ratio MRT/aTTT tended towards significance (P = 0.059). The MRT/aTTT ratio for the coarse diet was significanlty higher and closer to 1 than the fine diet ratio meaning that the aTTT and MRT were more similar for the coarse than the fine diet. One could speculate that the large particles from the coarse diet resided longer in the stomach (like the capsule) than the particles of the fine diet. The MaxRT/aTTT ratio was 0.97 vs. 1.19 for the fine diet and the coarse diet, respectively, indicating that on the coarse diet, the capsule tended to be even excreted sooner than the last titanium marker. Also, the higher MaxRT of the coarse diet (33.3 h vs 30.8 h) might indicate that some parts of the coarse diet are not passed as fast as the fine diet.

2.5.4 *Biological implications and conclusions*

In general, we could not prove any substantial effect of particle size on the transit characteristics obtained by the IntelliCap capsule. However, MaxRT values obtained through the second marker system (TiO_2) differed between diets. The MaxRT difference might not be big enough to cause any physiological consequences but indicates that particle size might affect the mechanics in the gut. One could state that particle size might have been undone due to chewing on the food although this was very unlikely. Wolves, the dog's wild ancestor Axelsson et al. (2013), are known to gorge feed (Bosch et al., 2015) and this is a characteristic still to be found in dogs. During this experiment, a similar behaviour was observed with all dogs barely chewing their food. Overall, analyses show that the titanium marker and the capsule differ more on the fine diet and less on the coarse diet. For the coarse diet, it could even happen that the capsule is excreted sooner than the last titanium marker. One could state that for a wild carnivore or a carnivore fed a natural diet (whole prey, coarse), the capsule could be an adequate reflection of how passage happens through the gut. However, for

artificial diets with fine particle sizes and less texture, the capsule might not be a representative marker for the gastrointestinal passage with the passage probably being faster than measured by the capsule.

Although we could only prove a small MaxRT difference between diets (33.3 h vs 30.8 h, coarse and fine diet, respectively), there is reason to believe that adding texture to a carnivore's natural diet influences transit time substantially. This might not only be through the factor particle size but even more likely through the addition of animal fibre (i.e. poorly digestible animal tissues ((glyco)protein-rich matter such as raw bones, tendons, cartilage, skin, hair or feathers)), which might play a more crucial role in guiding digestive processes such as transit time. Depauw et al. (2013) showed that feeding whole rabbit to cheetahs compared to supplemented beef resulted in a lower amount of putrefactive fermentation products and a better faecal consistency. The mechanism underlying the improved gut health was not completely clear, although it was speculated that the presence of more animal fibre in the whole rabbit-diet might have influenced gastric emptying, passage rate, motility and absorption. In dogs, it is shown that by the inclusion of plant-derived insoluble fibre in the diet, transit it is affected. According to Burrows et al. (1982) the inclusion of cellulose in a canine diet decreases total transit time. Pedreira et al. (2013) showed that the inclusion of 10 % insoluble fibre (sugarcane fibre) in a dog’s diet delays the gastric emptying and colonic filling time. It might be that animal fibre exerts similar effects on transit parameter as the plant-derived analog and that through extended gastric fill, satiety is prolonged (Pappas et al., 1989). However, more research is warranted concerning the effect of texture - by varying the dietary animal fibre content, or maybe equally important, the particle size of animal fibre in the diet - on transit parameters in carnivores.

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3. Are carnivore digestive separation mechanisms revealed on structure-rich diets?: Faecal inconsistency in dogs (*Canis familiaris*) fed day-old-chicks

Adapted from

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3.1 Abstract

Pronounced variations in faecal consistency have been described anecdotally for some carnivore species fed a structure-rich diet. Typically two faecal consistencies are distinguished, namely hard and firm versus liquid and viscous faeces. It is possible that a separation mechanism is operating in the carnivore digestive tract, as in many herbivore species. Six beagle dogs were fed two experimental diets in a cross-over design of 7 days. Test diets consisted of chunked day-old-chicks differing only in particle size (fine = 7.8 mm vs coarse = 13 mm). Digestive retention time was measured using titanium oxide (TiO_2) as marker. The total faecal output was scored for consistency and faecal fermentation profiles were evaluated through faecal short-chain fatty acid (SCFA) and ammonia (NH_3) analyses. A total of 181 faecal samples were collected. A faecal consistency dichotomy was observed with firm faeces (score 2-2.5) and soft faeces (score 4-4.5) being the most frequently occurring consistencies in an almost alternating pattern. Firm and soft faeces differed distinctively in fermentative profiles. The latter strengthens the hypothesis that a digestive separation mechanism is operating in the canine digestive tract that can be provoked by a structure-rich diet. Further faecal characterisation is however required in order to unravel the underlying mechanism.

3.2 Introduction

Separation mechanisms in the digestive tract that selectively retain either fluids or particles have been described in many herbivorous species such as ruminants, lagomorphs, rodents and some birds (Björnhag, 1981; Dittmann et al., 2015; Frei et al., 2015; Frei et al., 2017). Typically, the functional existence of these mechanisms is explained either with respect to a comparative delay or acceleration of plant fibre particles to, respectively, enhance their digestion or to rid the digestive tract of them quickly (Schwarm et al., 2008) or with respect to a washing of the particulate digesta by fluid in order to direct very fine particles, including microbes, in an aborad or orad direction (Müller et al., 2011). In mammalian hindgut fermenters the first principle often occurs when the time-consuming process of fibre fermentation is accounted for by selectively retaining the small, easy-to-ferment plant fibre particles and excreting the larger, coarse, more difficult-to-ferment particles more rapidly from the hindgut (Björnhag, 1981; Björnhag et al., 1984). Similarly, in some birds, this physical principle occurs with fluids and fine matter being retained in the caeca and coarse, large particles being excreted with the ordinary faeces (Björnhag, 1981; Björnhag, 1989; Frei et al., 2017). Typically, this results in longer retention times for the fluid fraction and shorter retention times for larger particles (Gasaway et al., 1975; Frei et al., 2017). In turkey (*Meleagris gallopavo*), this separation in particles leads to the presence of two faecal consistencies - solid vs liquid - with larger particles that tend to be excreted with solid excreta whereas the smaller ones are excreted in more liquid excreta (Frei et al., 2017) in which the protein level and microbial count is higher (Björnhag, 1989). Although not specifically studied to date, there is reason to believe that separation mechanisms are present in carnivores as well. Wolves (*Canis lupus*) fed whole prey produce two types of faeces, i.e. firm, hard faeces and dark, watery, loose faeces, as described by Floyd et al. (1978), Weaver (1993), Ruehe et al. (2003) and Jethva and Jhala (2004). The liquid faeces are considered non-collectable and therefore are not included in faecal analyses to evaluate the feeding ecology of wild wolves. Similarly, a discrepancy in faecal consistency has been observed when feeding cheetahs (*Acinonyx jubatus*) whole prey, with collectable faeces described as hard to soft and non-collectable faeces as viscous (Marker et al., 2003; Wachter et al., 2012). To our knowledge, the systematic occurrence of two faecal consistencies within a diet has not been reported in scientific literature in healthy domestic carnivores fed commercially prepared diets (from dry kibble diets to processed meat). Only Hill et al. (2011) observed that the water content of faeces and looser (watery) faeces, was higher in the afternoon than in the morning

in dogs fed canned diets containing texturised vegetable protein from soya in morning meals, which was attributed to the soy carbohydrates present in the texturised vegetable protein. Based on these reports, we speculate that the occurrence of two types of faeces might be an indication of a separation mechanism operating in the hindgut which might be linked to different substances in a heterogeneous carnivore diet. Examples of more recalcitrant substances are skin, hair, bone or collagen in whole prey (i.e., 'animal fibre' (Depauw et al., 2013)), which may have some analogies with the coarse or larger-sized, difficult-to-digest plant material consumed by herbivorous species. As in plant-derived fibre, more soluble and insoluble fractions can be distinguished within 'animal fibre', with collagen representing the soluble, smaller particles and fermentable fraction and substances such as hairs and bones as the more insoluble, coarser fraction (Depauw et al., 2012), which could provoke a possible separation in the hindgut as described above for the herbivorous species. Therefore, as a first step, we wanted to evaluate how the digestive physiology of the dog, as an example of a carnivore species, is affected when fed a whole prey diet. As particle size may impact the separation efficiencies (Dittmann et al., 2015) we included this as a dietary contrast in our study design (**Chapter 2**). Insight in the digestive physiology was obtained by monitoring faecal patterns and associations between faecal consistency with retention time and faecal fermentation profiles.

3.3 Material and methods

3.3.1 *Experimental design and diet (See **Chapter 2**)*

Experimental procedures were approved by the Ethical Committee of the Faculty of Veterinary Medicine of Ghent University (EC2015/45). Six adult beagle dogs (four females and two males) with an average (± standard deviation (SD)) body weight of 10.1 kg (± 1.1), a body condition score between 3 and 5 on a scale of 1 (anorexic) to 9 (obese), and aged between 2 and 7 y, were fed two test diets in a cross-over design of 7 d per period. Both test diets were based on exclusively day-old chicks (Kiezebrink Putten B.V., Hoge Eng Oost, the Netherlands) minced at a die size of 7.8 mm for the fine diet or 13 mm for the coarse diet (KOLBE AW 130 meat mincer). This was the largest contrast that could be obtained within the limitations of the available food processing equipment. It was assumed that this contrast in die size would create a sufficiently large contrast in particle size. Because of the limited duration of the trial, the diets were not adjusted for any potential deviations from nutrient guidelines, in order to keep the intervention simple.

In order to adapt the dogs to the chunked day-old chicks, a 3-wk dietary adaptation period was provided before the actual start of the trial. In the first week, chunked day-old chicks (13 mm) were gradually added to the routinely fed kibble diet (fulfilling maintenance energy requirements (MER) for adult laboratory dogs (NRC, 2006)).

In the consecutive two weeks, chunked day-old chicks were meal-fed (100 % MER) to maintain constant body weight. Only one dog was often reluctant to eat its whole meal whereupon refusals were offered again at a later time point of the day. After the adaptation period, the cross-over trial was executed with dogs being meal-fed once between 8 AM and 9 AM every day with each dog always receiving the same amount of food throughout the cross-over experiment hence avoiding differences in food intake between dietary treatments. All dogs had *ad libitum* water access and were weighed weekly. A total faecal collection was carried out for every dog during the cross-over trial (14 d). Each kennel was checked every 15 min day and night for defaecation events and the time was recorded of each defaecation.

3.3.2 *Patterns of faecal consistency*

Before collection, the faecal consistency was scored for every sample using the Waltham faeces scoring system (Moxham, 2001) based on visual appearance. The scoring scale runs from 1 to 5 with 1 being 'hard, dry and crumbly faeces' and 5 being 'watery diarrhea'. Half-

scores were used, giving a total of 9 possible categories. Faecal samples were weighed, frozen at –20 °C and dried afterwards at 60 °C to constant weight for determination of the dry matter (DM) content.

3.3.3 *Transit time (See **Chapter 2**)*

Mean retention time (MRT) and maximum retention time (MaxRT) were determined for each treatment by adding 2 g TiO_2 (VWR, International BVBA, Leuven, Belgium) per kg of diet on the fifth day of every test period. The marker was poured upon the diet per dog and was mixed manually and thoroughly with the diet to ensure homogenous distribution of the marker. Faecal samples collected from one day before TiO_2 addition until two days after the TiO_2 addition were used for Ti analysis. All samples were scored (see above), weighed and dried at 60 °C.

3.3.4 *Fermentation products*

In order to analyse the microbial fermentation products, fresh faecal subsamples were collected within 15 min of defaecation for every dog on the third and fourth day of every test period. After scoring the faecal consistency (see above), pH was measured with a calibrated portable pH meter (HI 99141, pH electrode probe HI 72911, Hannah Instruments, Belgium). Afterwards, a representative aliquot of faeces was collected from every sample for short-chain fatty acid (SCFA; including branched-chain fatty acids (BCFA)) and NH_3 analyses. All fresh faecal samples were stored at -20 °C until further analyses.

3.3.5 *Chemical analyses*

Dietary DM was determined by drying to constant weight at 103 °C. Ash content was determined by combustion at 550°C. Crude protein (6.25 × N) was analysed using the Kjeldahl method (ISO, 2005) and crude fat was analysed according to the Soxhlet method (with and without pre-hydrolysis of samples) (ISO, 1973). Crude fibre was analysed by acid-alkali digestion (ISO, 1981). Total fibrous matter and insoluble fibre were analysed according to the method of Cools et al. (2015). This method is based on the *in vitro* digestive simulation of Boisen and Fernández (1995) and Hervera et al. (2007) and resembles the total dietary fibre (TDF) analysis according to Prosky et al. (1985) with the difference that the fibre fraction obtained includes not only the plant-derived carbohydrate fraction (TDF) but also animal fibre (protein-rich). Titanium in faeces was analysed according to the method of Myers et al. (2004) (See **Chapter 2**). For determination of SCFA and NH_3, ca. 0.5-1.0 g faeces was added

to safe-lock tubes (2 ml; Eppendorf AG, Hamburg, Germany) containing 1 ml of a 0.0333 M H_3PO_4 solution (for SCFA) or 1 ml of 10 % TCA solution (for NH_3). The content of the tubes was mixed on a vortex for ca. 3 sec and weighed. The mixed samples were centrifuged at 15,000 rpm for 5 min at 4°C (Centrifuge 5417R, Eppendorf AG). The sample supernatant was analysed for SCFA (acetic, propionic, isobutyric, butyric, isovaleric and valeric acids) and NH_3 concentrations following Bosch et al. (2008).

3.3.6 *Calculations*

The MRT of TiO_2, the best single measure of rate of passage through the gastro-intestinal tract, was calculated according to Thielemans et al. (1978).

$$\text{MRT (h)} = \Sigma\, t_i\, C_i\, \Delta t_i \,/\, \Sigma\, C_i\, \Delta t_i$$

where C_i is the marker concentration in the interval indicated by time t_i (hours after marker administration) and Δt_i = the interval of the concerning sample:

$$\Delta t_i = ((t_{i+1} - t_i) + (t_i - t_{i-1})) / 2$$

Furthermore, the time of last marker excretion (MaxRT) (< 5% of the peak concentration) was determined for both treatments. Additionally, marker excreta concentrations were plotted over time with concentrations expressed as the percent of the marker peak concentration (Matsuda et al., 2015).

In order to explore any difference in marker excretion between 'firm' (score 1 to 3.5) and 'soft' faeces (score 4 to 5) (see above), the percent of the marker peak concentration was labelled firm or soft.

Frequencies of every single faecal score were calculated per diet. Second, the average number of defaecations per day and the average faecal score per day were calculated per dog and per diet. Faecal scores were plotted over time per dog for the whole trial in order to explore faecal consistency data. Furthermore, faecal score frequencies were visualized using histograms for both dietary treatments. Additionally, a subdivision in faecal scores was made to firm and soft as indicated above. The number of firm and soft faeces per day and the ratio soft to firm faeces were calculated per dog and per diet. The SCFA and NH_3 were expressed on a DM basis. Furthermore, BCFA (isobutyric and isovaleric acid) was expressed as the percentage of the total SCFA (Awati et al., 2006).

3.3.7 *Statistical analyses*

The effect of dietary treatment on faecal SCFA, NH_3, and DM concentrations and pH values was evaluated using a linear mixed effect model (lmer function of the lme4 package in RStudio) with dietary treatment, period and group (order of dietary treatments) as fixed effects and dog as a random effect. Additionally, the faecal score was included as a continuous fixed effect in the model. The interaction between faecal score and dietary treatment was also included in the model, except when not significant ($P > 0.10$), then the interaction was omitted from the model. Results are reported as regression estimates.

Pearson correlations were determined for the following relationships: DM concentrations versus faecal score; average faecal score per day versus average number of defaecations per day; the number of soft faeces per day, the number of firm faeces per day and the ratio soft faeces:firm faeces versus MRT and also versus MaxRT. Relationships were considered trends when $0.05 < P < 0.10$.

3.4 Results

All dogs remained healthy throughout the study. A general decrease in bodyweight was observed for all dogs throughout the cross-over trial (approximately 3 % bodyweight loss). All provided food was consumed every day. Only one dog showed reluctance to eat its whole meal at once. Refusals were offered again at a later time point during the day except during retention time testing on the fifth day of the first test period. Subsequently, on the fifth day of the second test period, this dog was offered the same diminished amount of food in order to compare test periods (356 g instead of 808 g). The chunked day-old-chicks contained 38 % amount of total fibrous matter and 26.2 insoluble fibrous matter (on a DM basis) (Table 1).

Table 1 Analysed components and calculated energy content of chunked day-old-chicks (See Chapter 2)

	Chunked day-old-chicks
Component (% of DM)[a]	
Dry matter (% as is)	24.9
Crude protein	57.3
Crude fat	22.7-26.4[b]
Total fibrous matter	38.0
Insoluble fibre	26.2
Crude ash	7.1
Crude fibre	2.5
Metabolisable energy (kJ/100 g DM)[c]	1672

DM = dry matter; [a] Unless otherwise stated; [b] Smallest value without hydrolysis, largest value with hydrolysis; [c] The metabolisable energy is the average of the values calculated by Atwater factors (16.7 × crude protein + 37.7 × crude fat + 16.7 × NfE) and the alternative predictive equation of the NRC (2006) with NfE (Nitrogen free extract) calculated as 100 - moisture% - crude protein% - crude fat% - crude fibre% - crude ash%

3.4.1 *Patterns of faecal consistency*

A total of 181 faecal samples were collected. Liquid faeces (≥ score 4) were collected as completely as possible. The DM content negatively correlated with faecal score ($R = -0.719$, $P < 0.001$). By observing faecal score patterns over time for every dog, a dichotomy of firm and soft faeces within individuals became obvious. Figure 1 shows individual faecal patterns

of two dogs included in the experiment. When faecal scores were expressed as a frequency per diet (Fig. 2), the same pattern occurred with the scores 2-2.5 and 4-4.5 being the most frequently observed scores. The average number of soft faeces per day, firm faeces per day, the ratio soft faeces to firm faeces can be found in Table 2.

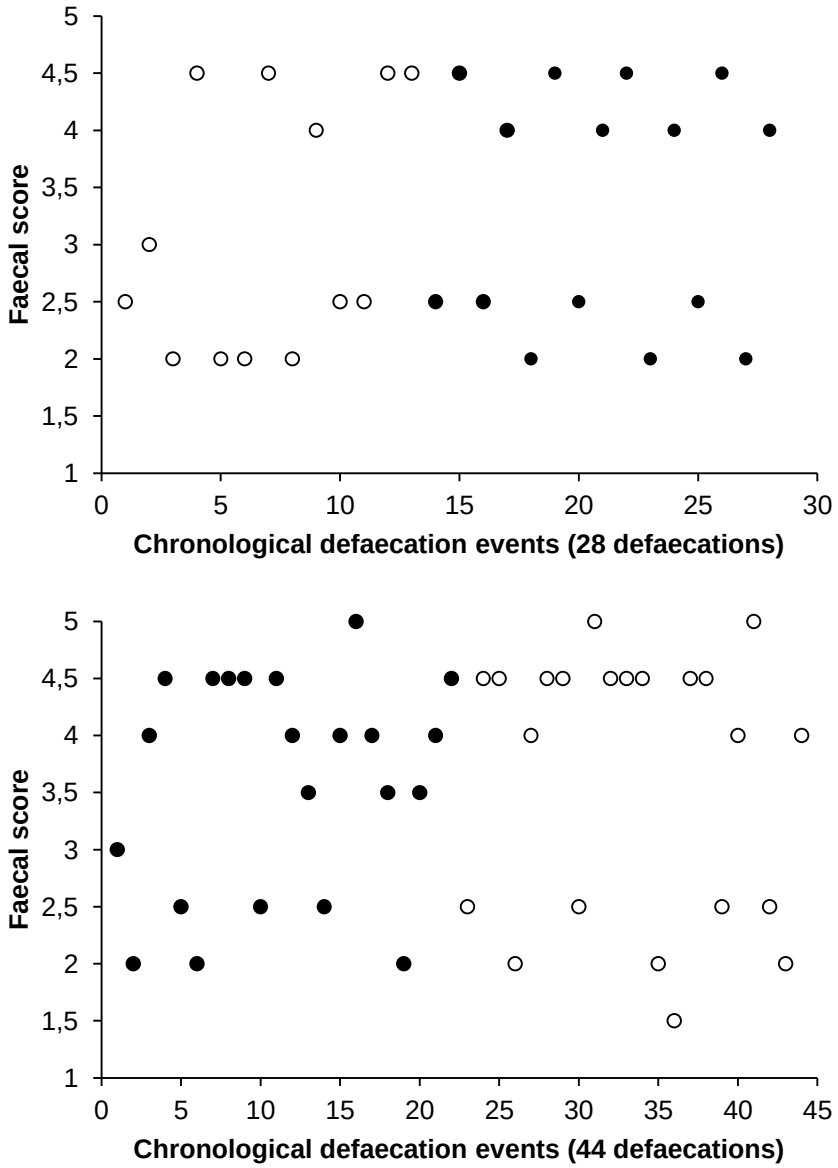

Fig. 1 Patterns of subsequent faecal consistency scores over a 14 d period of two beagle dogs Black circles = fine diet; white circles = coarse diet

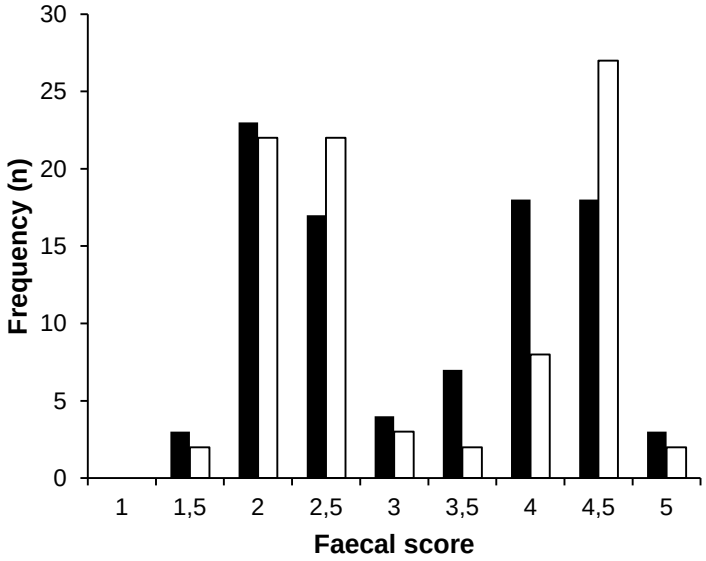

Fig. 2 Distribution of faecal score for two test diets Black bars = fine diet frequencies (n= 93 defaecations); white bars = coarse diet frequencies (n= 88 defaecations)

Table 2 Average daily number of defaecations, average daily faecal score, frequencies of faecal consistencies and transit times for 6 Beagle dogs fed a fine or coarse diets in a cross-over design

Parameter	**Fine diet**		**Coarse diet**	
	Mean	SD	Mean	SD
Defaecations/d	2.4	0.70	2.3	0.42
Faecal score/d	3	0.34	3	0.34
Soft faeces (n/d)	1.0	0.56	0.93	0.57
Firm faeces (n/d)	1.4	0.29	1.3	0.15
Ratio soft/firm	0.74	0.39	0.75	0.54
MRT (hrs)	19.5	5.0	22.0	3.8
MaxRT (hrs)	30.8	10.6	33.3	9.6

SD = standard deviation; n = number; MRT = mean retention time; MaxRT = maximum retention time

3.4.2 *Transit time*

The average TiO_2 recovery was 77.5% (SD = 10.8) without subdivision in dietary treatments (See **Chapter 2** for recoveries for both dietary treatments). The average MRT and MaxRT values are presented per diet in Table 2. Marker excretion patterns showed a single peak followed by a continuous decline without a difference between firm and soft faeces for all dogs on both diets, except for one dog that showed a recurrent peak of marker for soft faeces (Fig. 3a).

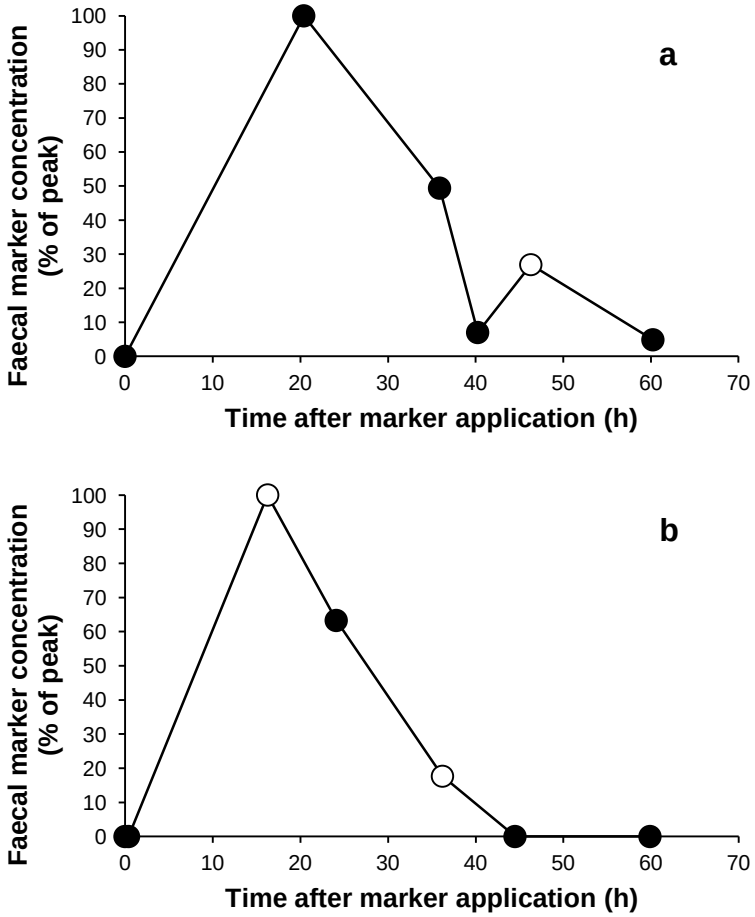

Fig. 3 Marker excretion patterns for two Beagle dogs Black circles = firm faeces (faecal score 1 to 3.5); White circles = soft faeces (faecal score 4 to 5); Graph a showing marker excretion pattern for one beagle dog on the coarse diet with two separate marker peaks; Graph b showing marker excretion pattern for one beagle dog on the coarse diet with one single marker peak

3.4.3 *Fermentation products*

Dietary treatment, period and group had no effect on DM, NH_3 and SCFA concentrations, except for butyric acid which was affected by dietary treatment ($P = 0.04$) and for which a tendency towards an interaction between treatment and faecal score was observed ($P = 0.06$). The pH values tended to be affected by dietary treatment ($P = 0.05$). Faecal score was not found to relate to variation in butyric acid and isovaleric acid. As faecal score increased, NH_3 ($P = 0.02$), acetic acid ($P < 0.001$) and valeric acid concentrations ($P < 0.001$) increased, whereas propionic acid ($P = 0.02$) and isobutyric ($P = 0.001$) concentrations decreased. Faecal pH decreased with faecal score ($P < 0.001$) (Table 3).

Table 3 Regression estimates (± SEM) for faecal DM, short-chain fatty acid (SCFA) and ammonia (NH_3) concentrations and faecal pH values from 6 Beagle dogs fed a fine or coarse diet in a latin square cross-over design. In the linear mixed effect model the fine diet was considered as the reference for Treatment and the diet order fine followed by coarse as the reference for Group

Parameter	Intercept	Treatment	Period	Group	Faecal score
DM (g/kg)	530.6*** (± 43.8)	-4.9 (± 11.3)	-2.8 (± 11.3)	-19.3 (± 19.9)	-54.0*** (± 5.4)
SCFA (mmol/kg DM)					
Acetic acid	39.6 (± 24.2)	-0.61 (± 6.3)	3.5 (± 6.3)	-9.9 (± 10.9)	19.0*** (± 3.0)
Propionic acid	62.3** (± 22.0)	-3.7 (± 4.6)	4.5 (± 4.6)	-2.0 (± 11.5)	-5.0* (± 2.2)
Butyric acid	49.4** (± 16.4)	-16.0* (± 7.9)	-1.0 (± 2.4)	-1.2 (± 4.9)	-5.1 (± 3.7)
Valeric acid	0.90 (± 0.62)	0.14 (± 0.20)	0.09 (± 0.20)	0.20 (± 0.20)	0.64*** (± 0.09)
Isobutyric acid	7.2* (± 3.7)	-0.49 (± 0.75)	1.4 (± 0.75)	1.3 (± 1.9)	-1.2** (± 0.36)
Isovaleric acid	8.6* (± 3.4)	-0.38 (± 0.80)	1.3 (± 0.80)	0.65 (± 1.7)	-0.23 (± 0.38)
NH_3 (g/kg DM)	2.0 (± 1.0)	-0.21 (± 0.26)	0.03 (± 0.26)	-0.29 (± 0.47)	0.30* (± 0.12)
pH	7.4*** (± 0.43)	0.18 (± 0.09)	0.12 (± 0.09)	0.13 (± 0.22)	-0.22* (± 0.04)

* = P < 0.05; ** = P < 0.01; *** = P < 0.001; Relationships were considered trends when 0.05 < P < 0.10

3.4.4 *Correlations*

Across dogs, the average number of defaecations per day tended towards a positive correlation with the average daily faecal score for the fine diet (R = 0.733; P = 0.097) and the coarse diet (R = 0.774; P = 0.071) (Fig. 4; Table 2), i.e. dogs with a higher frequency of soft faeces had a larger number of defaecations. The number of soft faeces produced per day tended to be negatively correlated with the MRT for the fine diet (R = -0.780; P = 0.067) as well as the coarse diet (R = -0.739; P = 0.093), i.e. dogs with a higher frequency of soft faeces

had shorter retention times (Fig. 5a). Similarly, the number of soft faeces produced per day was negatively correlated to the MaxRT for the fine (R = -0.898; P = 0.015) and the coarse diet (R = -0.886; P = 0.019). The soft:firm faeces was negatively correlated to the MRT for the fine diet (R = -0.887; P = 0.018) but only tended towards a negative correlation on the coarse diet (R = -0.735; P = 0.096) (Fig. 5c). Correlations between the soft:firm faeces and MaxRT tended to be negative for the fine diet (R = -0.807; P = 0.052) and were negatively correlated for the coarse diet (R = -0.853; P = 0.031). No significant correlations were found between the number of firm faeces per day and MRT (Fig. 5b) or MaxRT.

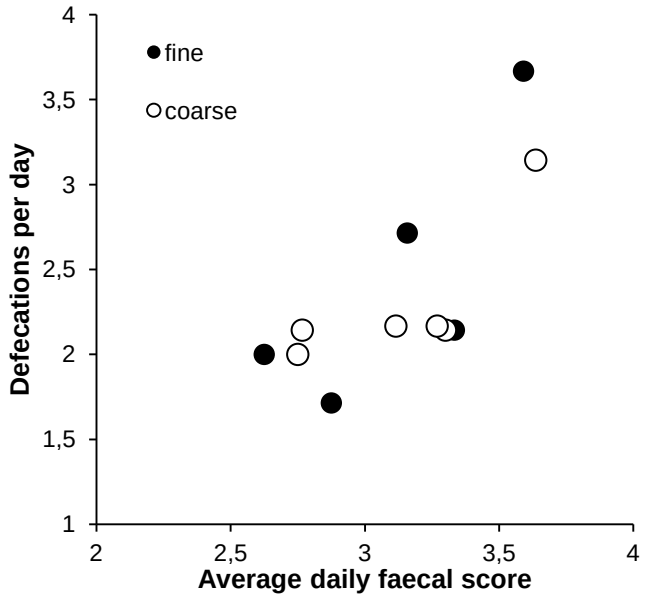

Fig. 4 Average number of defaecations per day vs the daily faecal score for two test diets Black circles = fine diet; white circles = coarse diet;

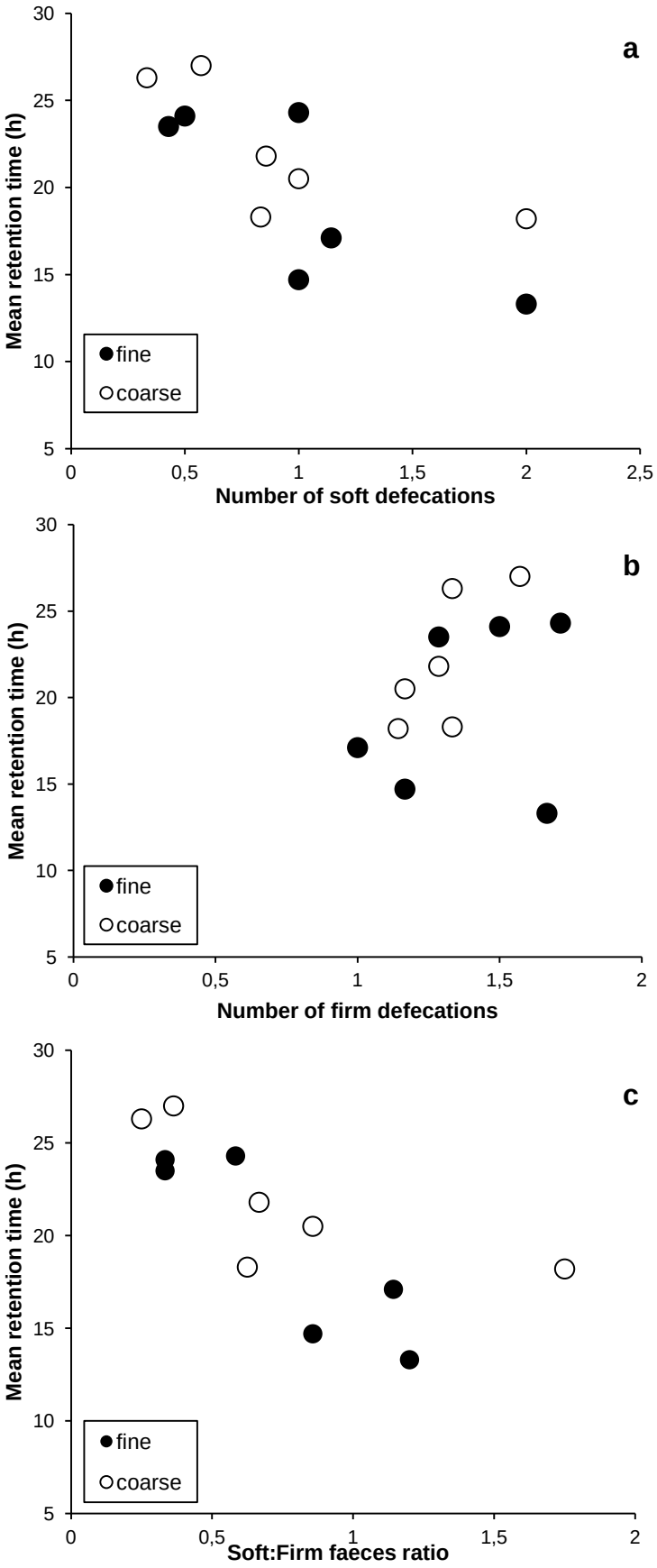

Fig. 5 Mean retention time vs the average frequency of soft and firm faeces and soft:firm ratio produced for two test diets Black circles = mean retention time on the fine diet; white circles = mean retention time on the coarse diet; n = 6

3.5 Discussion

This study provides a first insight in a possible digestive separation mechanism present in the hindgut of carnivores provoked by varying particle sizes with different fermentative properties in a whole prey-like diet. If a separation mechanism would be apparent, we expected it to result in the occurrence of two types of faecal consistencies with each a different retention and fermentation profile.

3.5.1 *Faecal consistency*

When fed the whole prey-like diet, each dog in this study showed two types of faecal consistencies with concomitant differences in DM concentration, namely hard, firm faeces with a score around 2-2.5 vs softer, more liquid faeces with a score around 4-4.5. The latter however did not differ between dietary treatments. When fed the coarser diet, we expected the faecal consistency duality to occur compared to the finer diet, where we expected the duality to be absent. Instead, we observed the two types of faeces for both dietary treatments. The main reason probably lies in the die sizes that were used to create the two particle sizes which was only 5.2 mm and too small to vary the dietary particle size sufficiently (**See Chapter 2**). One could state that particle size might have been undone due to chewing on the food. However, the dogs in this study tended to gorge feed, as does their wild ancestor the wolf (Axelsson et al., 2013), on the chick diet which makes this statement unlikely.

Although not quantified, the occurrence of two faecal consistencies is in contrast to the normal defaecation pattern of these dogs when kept on their traditional commercial dry kibble diet (Hill's Science Plan Advanced Fitness, 1570 kJ/100 g) (ADC, personal observation). Faecal consistency dichotomies have been described for wild carnivores in captivity. Wolves (*C. lupus*) and cheetahs (*A. jubatus*) both have been described as defecating 'collectable' (= firm, hard) and 'non-collectable' (soft, liquid or viscous) faeces when fed whole prey (Floyd et al., 1978; Weaver, 1993; Marker et al., 2003; Ruehe et al., 2003; Jethva and Jhala, 2004; Wachter et al., 2012). Additionally, it has been shown that the water content of faeces is higher in the afternoon than in the morning in morning-fed dogs consuming canned diets containing texturised vegetable protein from soya (Hill et al., 2011). To our knowledge, the intra-individual dichotomy of two types of faecal consistencies on a carnivorous diet has not been reported in any other studies than those for the wolf, cheetah and dog. A large number of studies investigated the effect of different diets on, amongst other factors, faecal consistency in various domestic and wild carnivores including the bobcat (*Felis rufus*), cheetah (*A.*

jubatus), tiger (*Panthera tigris*), jaguar (*Panthera onca*), African wildcat (*Felis lybica*), domestic cat (*Felis catus*) and domestic dog (*Canis familiaris*) (e.g. Vester et al., 2008; Vester et al., 2010; Hooda et al., 2012; Kerr et al., 2012; Kerr et al., 2013a; Kerr et al., 2013b; Kerr et al., 2013c; Kerr et al., 2014). However, authors did not specifically report on profound differences in faecal consistency when a carnivore was fed a specific study diet. It is possible that the intra-individual dichotomies in faecal consistency was not elicited by the specific diets in these studies, it was left unnoticed or it is not a common feature in carnivore digestive physiology. Careful recording of its absence as well as presence in future studies in other carnivorous species will allow further exploration of the variation in this aspect of digestive physiology. We postulate that in particular the presence of soluble and insoluble coarse substances in the whole-prey like diet is key in eliciting digesta fractionation by the gastrointestinal tract resulting in the dichotomy of faecal consistency. Whole prey contains substances that are coarse in texture (e.g. bones, tendons, cartilage, hair and feathers). Since wild carnivores will swallow chunks and pieces of different sizes from their prey, the particle size of the ingested food will vary naturally.

In this study, 42 % of the faeces (based on the frequency of defaecation, not on the amount) was liquid or soft. In wolves, it was reported that only 13% of the scats were liquid (Jethva and Jhala, 2004). Similarly, only 8% of cheetah scats were considered liquid (Wachter et al., 2012). Both render the impression that liquid faeces are produced to a lesser extent than firm faeces when on a whole prey diet, which is a more pronounced difference compared to our study outcome. However, it must be noted that our percentage of liquid or soft faeces reflects the faecal scores 4 to 5 whereas the percentages mentioned for wolf and cheetah only concern liquid faeces. In the wolf and cheetah feeding trials, several prey sizes were fed consecutively with carnivores being fasted up to 2 days before and after feeding a single prey (Floyd et al., 1978; Jethva and Jhala, 2004; Wachter et al., 2012). The faecal liquid percentages given above are an overall percentage across prey sizes. However, the number of firm faeces produced can differ between prey types fed with the number of firm faeces per kg of prey eaten decreasing as the size of the prey increases which implies that smaller prey consists out of relatively more indigestible material (Floyd et al., 1978). For example, for the wolf, Floyd et al. (1978) mentioned 87 % firm faeces per kg prey eaten for the groundhog (*Marmota monax*) and only 36 % firm faeces per kg prey eaten for the adult white-tailed deer (*Odocoileus virginianus*). Given the variation in intra-individual defaecation patterns in our six dogs, with ratios of soft versus firm faeces varying between 0.33 and 1.75, it appears

questionable whether a fixed ratio should be expected. Differences in frequencies between studies might just point to dietary differences, i.e. differences in fractions that are useful to be fermented might be separated. When feeding a diet with a lot of fermentable material, one would expect more soft faeces according to that hypothesis. The tendency towards a negative correlation between the daily number of soft faeces and the MRT and the negative correlation between the daily number of soft faeces and the MaxRT implies that at shorter overall retention times, more soft faeces were defecated (Fig. 5). Our individual dogs hence might have differed in the extent to which softer digesta components were either directly defecated, or retained in the colon for water re-absorption. Rolfe et al. (2002) mentioned that with a shorter transit time, the capacity to absorb water and electrolytes in the colon becomes impeded and leads to the production of softer, loose stools. However, it is possible that water and electrolyte absorption is not the strongest determinant for faecal moisture, instead higher fermentation activities due to a longer residence time in the colon are can be responsible for a higher faecal score (Macfarlane et al., 1998; Macfarlane and Macfarlane, 2003; Weber et al., 2004; Hernot et al., 2005).

3.5.2 *Characteristics of two types of faecal consistencies*

Differences in fermentation profiles between the observed faecal consistencies were present, which suggests gastro-intestinal separation of substances with distinct fermentation properties. As faeces were softer, NH_3, acetic acid and valeric acid concentrations were higher whereas propionic acid and isobutyric acid concentrations as well as pH values were lower compared to firmer faeces. The fibre type present in the experimental diets was exclusively animal fibre and thus protein-rich (total fibrous matter = 38.0 % of DM; insoluble fibrous matter = 26.2 % of DM). Faecal SCFA and ammonia concentrations were comparable to the levels found in domestic dogs fed commercial diets rich in plant-derived fibre (Bosch et al., 2009; Beloshapka et al., 2012). This suggests that the undigested parts of the chick diet can serve as a source for SCFA production as shown in humans and cheetahs (Macfarlane et al., 1992; Depauw et al., 2012; Depauw et al., 2013) with different animal based substrates that have different fermentative profiles (Depauw et al., 2012; Depauw et al., 2013). Based on the ratios acetic acid, propionic acid and butyric acid to total SCFA from our study and the ratios from in vitro fermentation of animal-based substrates (Depauw et al., 2012), collagen, cartilage and glucosamine-chondroitine were potentially substrates for fermentation in the undigested parts of the chick. The higher acetic acid concentration in the soft faeces type suggests more fermentation in the soft than the firm faeces type. It would typically be

attributed to carbohydrate fermentation, but can also be generated by protein fermentation (Macfarlane et al., 1986; Macfarlane et al., 1992). Ammonia and valeric acid concentrations, which are protein fermentation indicators (Macfarlane et al., 1986; Macfarlane et al., 1992), were higher for soft stools, suggesting a higher level of protein fermentation in softer faeces. However, such proteolytic fermentation is also associated with increased propionic acid and BCFA concentrations (isovaleric and isobutyric acid) (Macfarlane et al., 1992), which was not found in the present study and therefore do not support that acetic acid concentration was higher because of protein fermentation. The difference in the fermentative profile could however also be caused by a different uptake and utilisation of butyric or propionic acid by the mucosal cells of the large intestine due to differences in residence time and thereby absorption of SCFA across the mucosal barrier. Faecal pH decreased with faecal score, which is typically to be expected when SCFA including lactate are produced (Nakae and Elliott, 1965; Bergman, 1990). Yet, the only measured SCFA that increased in the soft faeces type was acetic acid, a weak acid (Bergman, 1990). Therefore, we suspect that the lower pH in the soft faeces type is caused by the production of lactate, a stronger volatile acid than the other SCFA. Lactate can cause a significant decrease in pH which can inhibit production of other SCFA, except for acetic acid that can be, under certain circumstances, high (Nakae and Elliott, 1965). Although animal fibre typically consists of indigestible proteins, substances such as chondroitin sulphate contain glucose chains (Scott, 1988) and may serve as substrates for lactate production (Vázquez et al., 2013). In many digestive systems, lactate is easily converted to propionate, with a prominent role for Bacteroidetes (termites: Schultz and Breznak (1979); humans: Ríos-Covián et al. 2016)). However, in cheetahs fed a whole prey diet, strains of Bacteroidetes were very low in numbers (Becker et al., 2014), which allows speculation that lactate concentrations might have been high in the caecum and lowered the pH in the absence of conversion to propionate. We therefore recommend to measure faecal lactate concentrations in softer faeces in future studies. If a separation mechanism would be apparent in dogs, we would expect the digesta fractions, i.e. soft and firm faeces, to transit differently through the intestinal tract based on other herbivore and avian species (Schwarm et al., 2008; Frei et al., 2015; Frei et al., 2017). However, no pattern of marker excretion differences between firm and soft faeces could be observed in this study except for one dog (Fig. 3a), indicating that a monophasic digesta movement may not always be the case. However, since this only occurred for one dog in one test period, this might be a coincidental observation. Nonetheless, future research to study fluid and particle digesta phases separately is of interest. Typical retention time measurements in carnivores are done with a particle

marker (Itoh et al., 1986; Bruce et al., 1999; Nelson et al., 2001). Also TiO_2 is insoluble and therefore will have followed the solid fraction of the digesta in this study (Bedford et al., 2016). Whereas it is common practice in studies on the digestive physiology in herbivores to compare the movements of fluids and particles in the digestive tract (Müller et al., 2011; Dittmann et al., 2015), it is rarely done in carnivores. Most likely, this is due to the impression that little differences are to be expected between the digesta phases, and hence such tests may have little physiological relevance. The comparison of fluid and particle marker however can yield insights into retention mechanisms.

3.5.3 *Possible gut mechanics underlying the faecal separation*

This study showed that dogs fed chunked day-old chicks produce two types of faeces, each with distinct fermentation profiles. These findings suggest that dogs have a separation mechanism in the gastrointestinal tract.

If we assume similar reasoning as in herbivores and keep in mind the analogies between plant and animal fibre, the colon and caecum could be involved. A typical strategy used in hindgut fermenters to account for the time-consuming process of plant particle fermentation is to selectively retain the small, easy-to-digest particles and to excrete the larger, bulky, more difficult-to-digest particles more rapidly from the hindgut (Björnhag, 1981; Björnhag et al., 1984). The chunked day-old-chicks used in this study contained high amounts of animal fibre with the insoluble fraction representing the more bulky material such as hairs and bones (Depauw et al., 2012; Depauw et al., 2013). We speculate that it is possible that the bulky, difficult-to-digest animal fibre was excreted faster from the colon and the more fermentable fraction (e.g. collagen) was retained longer in the hindgut. Bowland and Bowland (1991) evaluated the passage of prey components through the gastrointestinal tract of the serval (*Felis serval*) and the black-backed jackal (*Canis mesomelas*). They noted that after consumption of whole prey, the majority of hairs, bones and teeth were excreted in the first scats for both species which could imply that the more difficult-to-digest part of the prey item was excreted faster from the hindgut. The retained faecal bolus could be subject to more fermentation, hence causing an osmotic gradient for water to move towards the lumen of the colon or caecum, rendering softer stools (Weber et al., 2004). The question remains how the selective retention-excretion of particles could be established in the caecum and colon. The canine caecum is highly variable in size. In situ, the caecum of mongrel dogs (ca. 30 kg) has a diameter of about 2 cm and a length of about 6 cm. When straightened out it can reach up to 8 cm in length and 3 cm in diameter (Abd-El-Hady et al., 2013). Others state straightened out

lengths of 10 to 15 cm and 1-2 cm in diameter (Sarna et al., 1988) and 8 to 30 cm of length in general (Nickel et al., 1979). Variation probably stems from the use of different dog breeds and sizes. Additionally, Banta et al. (1978) reported that SCFA production in the caecum of dogs was highest compared to the other digestive compartments. The canine caecum also periodically generates giant migrating contractions (GMC's) which are believed to release digesta boluses into the colon (Sarna et al., 1988). When studying faecal descriptions of carnivores that do not possess a caecum, findings are contradictory. The ferret (*Mustela putorius*) does not have a caecum (Powers and Brown, 2012; McGrosky et al., 2016) and typically only produces hard stools when fed whole prey (Powers and Brown, 2012). Additionally, when the retention patterns of a fluid and a particle marker were evaluated e.g. in giant anteaters (*Myrmecophaga tridactyla*), insectivores with a simple digestive tract without a caecum, there was no evident difference between the markers (Gull et al., 2015). However, the panda (*Ailuropoda melanoleuca*) has no caecum and when fed a diet based on bamboo, sugar cane and gruel, they produce normal and mucous stools at various intervals (Mainka et al., 1989), which seems to contradict the hypothesis that a caecum is a prerequisite for a separation mechanisms in the hindgut, and therefore requires further study. In addition, when making the comparison between herbivores and/or birds vs carnivores, one must keep in mind that quantitative features of the large intestine considerably differ. When for instance the length per kg bodyweight of the large intestine and caecum of turkeys and wolves (as ancestor of the dog) is compared, one can observe 2.73 cm/kg bodyweight for the large intestine and 5.73 cm/kg bodyweight for the caecum in turkeys (Leopold, 1953) which clearly differs from the values seen in wolves: 1.96 cm/kg bodyweight and 0.33 cm/kg bodyweight, for colon and caecum respectively (McGrosky et al., 2016).

Although faecal moisture and consistency should not be linked to upper gastrointestinal transit (e.g. gastric emptying time) (Weber et al., 2002), it could be that the stomach plays a regulating role in the dispersal of coarse and fine particles. It is known for dogs that objects of different size differ in the time at which they leave stomach. Once exceeding a threshold of ca. 5 mm diameter non-food particles are retained in the stomach until the interdigestive migratory myoelectric complex (IMMC) occurs, which drives large particles towards the duodenum (Itoh et al., 1986; Wyse et al., 2003). Hence, in this way, one would expect hair, bones and teeth in whole prey to reside longer in the stomach which might cause separation of dietary fractions. As such, dense bolusses of recalcitrant substances are released from the stomach in the interdigestive phase. These dense packages might not have undergone similar

digestion and fluid mixing as the average food particle and might have ended up in the hindgut after which they could be expelled immediately. The latter scenario, however, is in contrast with the findings of Bowland and Bowland (1991) (see above), reporting that substances such as teeth and bone are excreted in the first scats. Careful recording of the time sequence in which the two types of faeces occur after feeding, with a fasting period before and after feeding, should render more clarity in this matter.

It could be that the faeces differ in consistency due to a different retention time in the colon for reasons related to behaviour. Dogs might have retained their faeces in the colon/rectum hence enabling more fermentation (Weber et al., 2004) or more water and electrolyte absorption (Rolfe et al., 2002). However, one would not expect the faecal discrepancy to occur in an almost alternating pattern. Similarly, relating the faecal dichotomy to the activity pattern of dogs, i.e. diurnal rhythm, would not explain the alternating pattern that occured independently of the time of day.

In conclusion, a distinct faecal consistency pattern was observed in dogs fed whole-prey like diets which provoked by a hitherto unknown separation mechanism in the gastrointestinal tract of the dog. The distinct difference in fermentative profile between the soft and firm faeces supports this observation. Further characterisation of soft faeces in terms of microbiome, protein content and animal fibre levels is warranted as well as the passage analysis of fluid and particle digesta phases through the gastrointestinal tract to understand the specific mechanics underlying the faecal consistency duality.

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General discussion

1. Introduction

Carnivore body size seems a strong driver of carnivore feeding strategies in the wild with co-evolved adaptations in the digestive physiology of terrestrial carnivorous mammals. Feeding strategies, as in *what* and *how* a carnivore consumes, are currently only partially approached by allometrically scaling to carnivore body size. Typical prey species preyed on by carnivores have been studied extensively for numerous carnivore species (e.g. the lion (*Panthera leo*), the spotted hyena (*Crocuta crocuta*), the tiger (*Panthera tigris*), the leopard (*Panthera pardus*), the cheetah (*Acinonyx jubatus*) and the African wild dog (*Lycaon pictus*), reviewed by Hayward and collaborators (Hayward and Kerley, 2005; Hayward, 2006; Hayward et al., 2006a; Hayward et al., 2006b; Hayward et al., 2006c; Hayward et al., 2012)). The corresponding prey sizes of carnivores have been scaled empirically to predator size in which the size of the predator drives the choice for a specific prey size (Carbone et al., 1999; Carbone et al., 2014). Below the cutoff value of ca. 20 kg of predator mass, carnivores typically consume prey much smaller than themselves. Above the 20 kg treshold, carnivores will opt for a similar sized prey (Carbone et al., 1999). Hence, for the '*what*' carnivores consume, the predator mass seems to be a strong determinant rendering two functional carnivore groups (small vs large). However, the '*how*' carnivores consume and the possible implications for digestive physiology have not been considered as a result of predator mass so far, and hence have not been adressed allometrically. Only species-specific reports in the literature raise the impression that the functional group of *large carnivores* (hunting large prey) *selectively* ingest *large amounts* of highly digestible prey parts alternating with periods of either digestion and inertia or famine (i.e. *low kill frequencies*) which possibly has led to certain adaptations in metabolic pathways (e.g. the wolf (*Canis lupus*) (Bosch et al., 2015)). On the other hand, catching and consuming small prey, by *small carnivores*, appears to go together with a *complete consumption* of prey with a *regular* food intake, which in turn might have led to the ability of losing certain metabolic pathways (e.g. the wildcat (*Felis silvestris*) (MacDonald et al., 1984; Morris, 2002; Bradshaw, 2006)).

Chapter 1 tried to clarify to which degree carnivore body size might have determined carnivore feeding strategies by modelling the feature kill frequency, an important player when considering carnivore feeding strategies and ecosystems (Holling, 1959; Vucetich et al., 2011), and allometrically scaling it to carnivore body size. Since current knowledge on field kill frequency data is lacking due to methodological difficulties, one has to rely on kill

frequency estimates. Previous estimates of carnivore kill frequency have been scaled to carnivore body mass (Peters, 1983; Vézina, 1985). Typically, these estimates are based on two pillars, i.e. prey size and carnivore energetic requirements, often without consideration of carnivore functional groups, i.e. differing feeding strategies. The model developed in this dissertation (**Chapter 1**) accounted for the following factors: carnivore mass, prey mass, carnivore specific maintenance energy requirements and metabolisable energy in prey, hunting pack size, selective feeding and carnivore gut capacity with adaptations to carnivore functional groups. This model tried to elucidate whether a decrease in kill frequency occurs with an increase in carnivore body size, taking into account carnivore functional groups, and whether apart from species-specific reports in literature, the body size driven feeding strategy holds to be true for a broad range of carnivores.

Furthermore, this dissertation tried to offer a linkage between the empirically established relationship of body size versus kill frequency (**Chapter 1**) and the actual digestive physiology occuring when feeding whole prey diets to carnivores (**Chapter 2 and 3**). The kill frequency model established in this dissertation is closely associated with gastrointestinal features such as gut capacity and passage of the diet through the gut. Inevitably, when considering these gastrointestinal features for free ranging carnivores, one has to rely on the few reports in literature that assessed gut capacity (i.e. stomach capacity) (e.g. the lion (*P. leo*) (Smuts, 1979; Packer et al., 1990)) and assume a certain passage through the gastrointestinal tract. Therefore, as a first step, gastrointestinal passage through the gastrointestinal tract was tested on a whole prey diet (**Chapter 2**) in order to better understand what drives these processes. By doing so, **Chapter 2** aimed at testing all passage parameters (i.e. gastric emptying, small intestinal transit, colonic transit and total transit) by varying the level of 'structure' in a whole prey diet in the domestic dog (*Canis familiaris*). There is an extensive amount of knowledge on passage rates in domestic dogs and other carnivores but with the majority of test diets consisting of traditional kibble diets or processed meats (Wyse et al., 2003), which differs from the heterogeneity present in whole prey. In conclusion, this dissertation tried to offer more insights in how a feeding strategy comes about 'externally' (kill frequency modelling, **Chapter 1**) and 'internally' (passage rates, **Chapter 2**, and other faecal characterisation, **Chapter 3**).

2. Is carnivore kill frequency a body size driven feature?

2.1 Model building: a path to generalisation

The feature kill frequency (x kills/predator/time unit) can be considered fundamental in the study of predator-prey interactions, not only to gather further insights in terrestrial ecosystems (Holling, 1959; Vucetich et al., 2011) but also, by allometrically scaling to predator size, to explore the existence of carnivore functional groups, i.e. the existence of a feeding strategy 'dichotomy'. Predator kill frequency has been previously modelled by Peters (1983) and Vézina (1985) and scaled to predator body size. Modelling of kill frequency (KF) typically occurs by, simply stated, dividing the energetic requirements of the predator (or the daily food intake) by the prey size. The general finding resulting from these KF scalings is a decrease in KF with a predator size increase, i.e. KF (prey/day) = $3.0\ M^{-0.47}$(Peters, 1983) and KF (prey/day) = $28.8\ M^{-0.427}$ (Vézina, 1985) (with M = predator body mass). As such, this outcome seems to abide by the hypothesis put forward in this dissertation (**Chapter 1**) that a functional dichotomy in the feature KF between predators exist based on body size, i.e. low kill frequencies for large predators vs high kill frequencies for small predators. However, one has to be careful by drawing such conclusions based solely on KF model outcomes. Instead, since KF model outcomes are strongly dependent on the range of predators included in datasets and the assumptions made concerning energetic requirements, one should carefully consider the methodology used in order to come to kill frequency estimates and its allometric scaling to predator body size for carnivores of the mammalian order of Carnivora.

The model of Peters (1983) as well as Vézina's model (1985) equals KF to daily ingestion rate (I, in kg/animal/day) divided by prey size (M_{prey}). The databases used by both authors are not solely restricted to mammalian carnivores but include avian carnivores as well. Both authors equal the daily ingestion rate (I) to an estimation of the scaling of energy requirements derived from caloric intake data of mainly captive animals from Farlow (1976). Additionally, the authors take into account that a single predator species can opt for a range of prey sizes and hence include the proportion of a certain prey size in the overall prey intake. Both models therefore include the frequency of occurrence of size class *i* (F_i) with *i* the position of class *i* with respect to the mean size class (*i* = 0) and $M_{prey(i)}$ being the midpoint of size class *i*: $KF = \Sigma\ I\ F_i / M_{prey(i)}$. As such, it seems that analyzing kill frequency as a function of carnivore body size in order to unravel the existence of carnivore functional groups within the mammalian

order of Carnivora, and even with a close focus on vertebrate feeders (see below), required new attention.

In **Chapter 1**, a new kill frequency model was developed taking into account the focus on mammalian carnivore 'vertebrate'-feeders and the important consequence of the carnivore-prey size relationship: carnivores taking prey whose mass exceeds their intake capacity can feed selectively on their prey, using only parts of increased energy density such as organs and muscle meat (Hornocker, 1967; Bowland and Bowland, 1991; Stahler et al., 2006; Bosch et al., 2015) whereas carnivores that kill relatively small prey will consume their prey entirely (Mills, 1996; Bothma and Coertze, 2004; Anwar et al., 2011) (Fig. 1).

In order to build a carnivore vertebrate-feeder database, only carnivores for which the diet consisted of more than 50 % vertebrate prey were included (Wilman et al., 2014). One can argue that in order to study functional dichotomies within the mammalian order of Carnivora, one must include all dietary groups such as insectivorous (e.g. aardwolf, *Proteles cristatus*) or omnivorous feeders (e.g. brown bear, *Ursus arctos*). Clearly, vertebrate and invertebrate foraging strategies cannot be compared in terms of search and feeding time, and these carnivores were therefore omitted from the dataset. Although carnivore species that prey largely on invertebrates were omitted from the database (Wilman et al., 2014), some carnivore species labeled as preying majorly on vertebrates showed average prey weights based entirely on invertebrates. The latter causes model kill frequencies that reach typically extremely high values and have to be interpreted with the feeding style and spatial distribution of invertebrate prey in mind, where a large number of individuals of social insects can be harvested in one single attempt.

The additional effect of social hunting was accounted for by dividing, for each species, M_{prey} by the average pack size ($N_{pack)}$. Kruuk (1972) and others (Fanshawe and Fitzgibbon, 1993; Creel and Creel, 1995; Creel, 1997; Funston et al., 2001; Carbone et al., 2005) suggested that pack hunting may have a share in subduing large prey since hunting by solitary hunters of similar-sized prey would not be successful. Hence, the subdued prey (M_{prey}) by a pack of carnivores needs to be corrected for the pack size (N_{pack}) in order to study individual prey intake (see below). Many literature reports describe pack size as is, without differentiation of age or functional classes. Often, during field studies in which determining pack size is typically executed over a wide time span, pack size can change during the study as young can enter the group and young and adults can be lost (Mills and Gorman, 1997). Therefore, field studies often are obliged to arbitrarily define pack size as a weighted average of pack

members. As such, the N_{pack} data obtained in this dataset are of an averaged kind, with the preference, if reported, of the pack size number excluding pups.

Carnivore specific maintenance energy requirements (Q_{pred}) were assessed using field metabolic rates (FMR) of free ranging carnivores (Nagy et al., 1999). Calculating intake for carnivores and thereby kill rates in this manner is advantageous since data are based on exclusively free ranging animals rather than mainly captive animals (Farlow, 1976; Peters, 1983; Vézina, 1985), and derive physiologically from energy expenditure and not from intake (as in the Farlow dataset used by Peters (1983) and Vézina (1985)). Therefore, this approach allows introducing gut capacity as an additional factor (see below), which is not possible if the energy requirement data is derived from a dataset (food intake) that already inherently reflects this constraint.

Following the FMR power function for carnivorous feeders of 2.23 kJ $M_{pred}^{0.85}$ d^{-1} from Nagy et al. (1999), the Q^{pred} in the dataset of **Chapter 1** was scaled to carnivore mass as 791kJ $M_{pred}^{0.85}$ d^{-1}. One must hereby acknowledge that Q_{pred} is based on the assessment of FMR by scaling it to body mass. Next to body size, other factors such as taxonomy, diet, habitat and season may determine the FMR of animals. Although, body size seems to be the strongest determinant of metabolic rate (71 % of the variation in FMR) and thereby declares most of the variation found in FMR (Nagy et al., 1999). In order to give only one example of how the use of FMR could be of more ecological relevance, the FMR was measured for six African wild dogs (*L. pictus*) and averaged 15.3 megajoules per day, which was¸ due to their high hunting costs, 25 times higher than the estimated basal metabolic rate (Gorman et al., 1998).

Possibly the most important adaptation in the KF model is the incorporation of consequences associated with carnivore functional groups, i.e. the duality of incomplete vs complete use of prey and whether or not a carnivore opts for selectivity feeding (see above). The main question in this matter was how to assign a certain carnivore to a functional group. One could opt for the 20 kg of carnivore mass cut off point above which carnivores are more large prey-feeders and below which small prey-feeding typically occurs (Carbone et al., 1999). However, one factor that was considered more decisive in the 'choice' for a functional group was the gut fill or gut capacity. The question of how selectively and how much a carnivore will consume will be constrained by the gut capacity (Chakrabarti et al., 2016) in relation to the pack corrected prey size (iMprey). The biomass consumed (per collectable scat) increases asymptotically with prey weight: biomass consumed (per collectable scat) reaches a ceiling at large prey weights (Wachter et al., 2012; Chakrabarti et al., 2016). Gut fill (among other

factors), as a physiological constraint, therefore limits the biomass intake at larger prey sizes. Instead of basing the division in carnivore functional groups solely on carnivore body mass, carnivores were further parametrized with gut capacity data. Hence, this model included the division of predators into those where iM_{prey} < 1% of C (i.e., predators mainly preying on insects), those where 1% of C < iM_{prey} < C (or 'small prey predators'), and those where C < iM_{prey} (or 'large prey predators' who cannot consume their average prey in one meal). Therefore, as long as the mass of an individual prey item (M_{prey}) is smaller than C, the iM_{prey} is the main driver of KF in this model. As soon as iM_{prey} is larger than C, however, C becomes the factor limiting KF. The gut capacity estimates were obtained from maximal gastric capacity data that were used to establish the allometric relationship for all carnivores included: $C = 0.09\ [0.06;0.14]\ M_{pred}^{1.19\ [1.07;1.30]}$. Typically, in herbivores, gut capacity estimates are obtained by measuring the total content of the gastrointestinal tract (Parra, 1978; Müller et al., 2013). The carnivore estimates used in this dissertation stem from the maximal stomach capacity reported in literature. Nonetheless, these estimates can be considered reliable since the carnivore stomach acts as a 'batch reactor' from which food is dispersed, hence playing a determining role in the maximal contents that can be sustained in the gastrointestinal tract (Hume, 2002). In the establishment of the allometric relationship, C estimates did not account for instance for the effects of social status in pack hunting-carnivores on prey intake (e.g. the wolf (Peterson and Ciucci, 2003)). However, given the fact that a large pack hunting carnivore such as the wolf will hunt fairly large prey, the amount of food available for the pack will be more than what the pack is able to consume (Peterson and Ciucci, 2003). As such, a similar C for pack hunting species will not provoke large biases in the estimation of KF.

Together with the considered effects of selective feeding on the energy content in prey and edible portion of prey, it becomes clear that when one tries to model a feature such as kill frequency, numerous assumptions and generalisations have to be made. Although the model building starts with a strong species-specific literature base (e.g. prey size, gut capacity, FMR, pack size) one has, in order to describe broad carnivore trends, to rely on the predictive capacity of the allometric relationship KF with body size. The most important disadvantage of body size scaling that is claimed is the loss of precision in order to obtain generality (Benedict, 1938) which holds truth: general descriptions will always be less accurate than species-specific or individual findings (Peters, 1983). However, as stated by Peters (1983): *"One cannot dismiss a theory because it is imprecise. Theories are rejected because they*

predict less well than their competitors. In most cases, there is no competing theory of equal generality, and so the criticism is empty unless it proposes that a number of specific theories will make the same predictions more accurately and more precisely[sic]." The criticism that one could make for the KF model established in **Chapter 1** is that the dependent variable 'kill frequency' is not a field observation that was empirically scaled to carnivore body mass. Instead, kill frequency was calculated, nevertheless partly based on field observations, and scaled to body size. However, obtaining field observations for every carnivore included in the dataset, is rather impossible. Compared to other KF models (Peters, 1983; Vézina, 1985), without claiming any superiority, this model included as a first the consequences associated with carnivore functional groups, which in its turn might have added to the KF model precision.

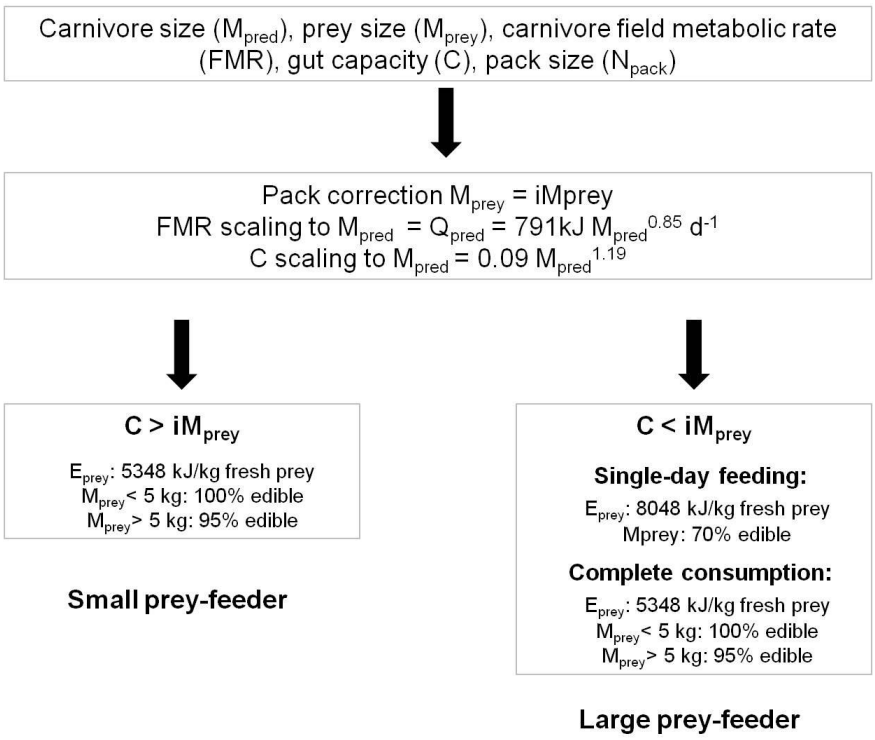

Fig. 1 Summary of kill frequency model parameterizations and modifications
KF = Q_{pred} / E_{prey} with KF = kill frequency, Qpred = carnivore specific maintenance energy requirements and Eprey = metabolisable energy in prey; E_{prey} of 5348 kJ/kg fresh weight obtained from Plantinga et al. (2011) and E_{prey} of of 8048 kJ/kg fresh weight obtained from Bosch et al. (2015); Edible biomass division of 100%, 95% and 70% based on Mills (1990), Stander (1992) and Caro (1994).

2.2 The variable small prey-feeders and body size driven large-prey feeders

2.2.1 *General differences between small and large prey-feeders*

Considering the predator-prey relationship established for the whole carnivore dataset in **Chapter 1**, prey mass scaled to carnivore mass with a scaling exponent larger than 1.0 (Table 1). Compared to other predator-prey scalings (Peters, 1983; Vézina, 1985; Carbone et al., 2014), these findings seem to largely coincide, all with scaling exponents exceeding 1.0 (Table 1): an overall increase in the mean prey mass with predator body mass. The impact of pack correction on the Mpred-Mprey relationship in the model approach in **Chapter** 1 was not distinct (Mprey = $0.03\text{Mpred}^{1.56}$ versus iMprey (pack corrected) = $0.03\text{Mpred}^{1.60}$); however, the majority of carnivore species in the dataset were solitary hunters. Lamprecht (1978, 1981) suggests that subduing and killing large prey might not be the most important objective of group hunting. Defending kills against competitors and other factors such as predation risk and intrasexual competition might exert stronger driving forces on the pack size number (see 2.2.2). However, there does not seem to be a particular consistent function for pack hunting for every single species; this can differ depending on the social or ecological situation (Lamprecht, 1981). Nonetheless, correction for pack size was considered essential in order to contribute to more accuracy.

Table 1 Comparison of predator-prey size scalings according to aM_{pred}^{b} in different datasets

Dataset/Taxon	N	a (95% CI)	b (95 % CI)	Reference
Mammalian terrestrial carnivores	74	0.03 (0.01;0.07)	1.56 (1.17;1.96)	Chapter 1
Mammalian terrestrial predators	270	0.007(0.004; 0.010)	1.05 (0.90; 1.20)	Carbone et al. (2014)
Terrestrial carnivores	44	0.085	1.18	Vézina (1985)
Terrestrial predators	49	0.109 (0.003;4.03)	1.16(0.87;1.45)	Peters (1983)[a]

Dataset of Chapter 1 is restricted to the order of Carnivora and focussed on vertebrate prey-feeders; dataset of Carbone et al. (2014) is not restricted to the order of Carnivora; dataset of Vézina (1985) includes birds, amphibians, lizards and mammals; dataset of Peters (1983) includes birds and mammals; [a] Scaling of Peters (1983) is based on the at that time unpublished data of Vézina.

In the predator-prey mass scaling in the model (**Chapter 1**), the 20 kg of predator mass cut-off point where predators switch from small to large prey-feeding (Carbone et al., 1999) seems to occur as well although some small carnivores seem to opt for large prey and vice versa. When considering our division in carnivore functional groups, i.e. large prey-feeders = $iM_{prey} > C$ and small prey-feeders = $1\%C < iM_{prey} < C$, this finding seems to be present as well: the majority of small prey-feeders is below, and of large prey-feeders above a body mass of 10-20 kg (with both linear scalings to body mass), and hence might be the actual biological driver of the observed 20 kg threshold. Yet, notably both feeding types occur across the whole body size range (Fig. 2). One conclusion that can be drawn from this finding is that whether or not carnivores catch prey larger or smaller than their own intake capacity, this is not necessarily driven by their own body size, i.e. some small carnivores seem to opt for a large prey-feeding strategy but by ecological conditions such as prey abundance and availability (Bonesi, 1996).

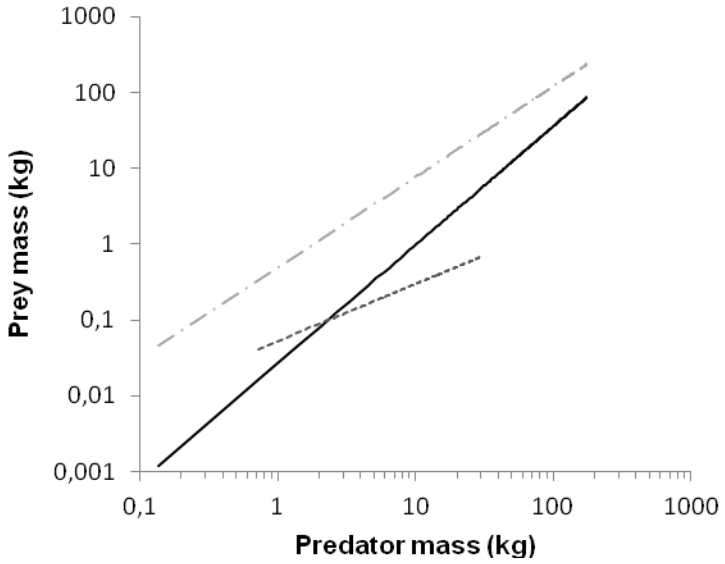

Fig. 2 Comparison of the scaling of prey mass (M_{prey}) with predator mass (M_{pred}) between the whole dataset and two functional carnivore groups (small prey-feeders and large prey-feeders) from Chapter 1 The solid black line represents the whole dataset scaling ($0.03\ Mpred^{1.56}$); the dark grey dotted line represents the small prey-feeder scaling ($0.05\ Mpred^{0.81}$); the light grey dotted/dashed line represents the large prey-feeders ($0.5\ Mpred^{1.19}$)

Kill frequency model outputs render similar findings. Although the general output for the whole body mass spectrum is a negative scaling of KF to carnivore bodymass, similarly to previously reported KF estimates (Peters, 1983; Vézina, 1985) (Table 2), this relationship did not exist for small prey-feeders. Indeed, the group of small prey-feeders is of a variable kind

which mainly results from preying on a broad spectrum of small prey, without constraints of gut capacity. Additionally, the small prey-feeder group includes carnivore body sizes exceeding the 20 kg carnivore body size treshold (Carbone et al., 1999). Some prominent examples of small prey-feeders in the mid-to-large sized spectrum can be sought in species-specific literature reports. The Ethiopian wolf (*Canis simensis*), with a bodymass of 15 kg on average, hence mid-sized, pursues prey with an average prey weight of 0.2 kg entirely based on small rodents and is therefore not limited by its gastric capacity. This renders a KF output of 44 kills per day, hence suggesting this carnivore to be an adherent of the small prey-feeding functional group. Indeed, the species is endemic to the Ethiopian highlands were it feeds exclusively on diurnal small rodents (Ashenafi et al., 2005; Marino et al., 2009). The red wolf *(Canis rufus)* with a bodymass of 30 kg, exceeding the 20 kg threshold, has a KF of 2.2 kills per day. The red wolf's 'high' KF value results from preying mainly on small rodents, lagomorphs and on occassion medium sized mammals such as the white-tailed deer *(Odocoileus virginianus)* (McVey et al., 2013) resulting in a rather low average prey weight that does not exceed gastric capacity, which in turn makes it a small prey-feeder despite its large body size. Generally, turning to small prey might be explained by several options: low availability of large prey in their habitat (cf. Ethiopian wolf), a lack of sociality in these species that otherwise would help to overcome large prey (Lamprecht, 1978), the constraints of hunting a large body sized prey with the risk of getting hurt (Packer et al., 1990; Funston et al., 2001), or the absolute energetic advantage (Carbone et al., 2007). In contrast to the functional group of small prey-feeders, the large prey-feeders did exhibit a negative scaling of KF to carnivore body size (Table 2). One major factor causing this linear scaling is the constraint of gut fill. Gut capacity will limit prey intake whenever this prey exceeds the gut capacity. Although this pattern was only expected above a 20 kg threshold (Carbone et al., 1999), this group is not restricted to large-body sized carnivores and occurs across the whole carnivore body size spectrum. Examples of small body-sized carnivores that opt for large prey-feeding strategy can be found in the Mustelid family. If the ermine or stoat *(Mustela erminea)* is sifted out with an average M_{pred} 0.24 kg, the M_{prey} turns out to average around 0.76 kg, which greatly exceeds its C, hence the KF is expected to be low. However, one important consideration in the handling of prey that exceeds the instantaneous intake capacity, is whether or not prey is consumed once until C is reached and left behind, or the prey is 'guarded' and fed on for several days. Both options were considered in the KF modelling: KF estimates were or based on a single-day feeding on their prey (in a selective mode, i.e. eating the amount of C at 8048 kJ/kg) or a complete consumption of their prey (in a non-selective

mode, i.e. with 95% consumption at 5348 kJ/kg) (See also 2.2.2). Considering the first option, KF would be 1.6 kills/day, the second option would render a KF of 0.06 kills/day. The average Mprey is based on publications where hare *(Lepus sp.)* is claimed to be the most frequently hunted prey (McDonald et al., 2000). Such large carcass cannot be eaten at once and indeed, Vander Wall (1990) mentions that also mustelids can show hoarding behaviour in order to preserve carcasses. As such, the KF outcome of complete consumption of prey over several days seems to abide with observations from the field.

Table 2 Comparison of predator-kill frequency (KF) scalings according to aM_{pred}^{b} in different datasets

Dataset/Taxon	Functional group	KF data	N	a (95% CI)	b (95 % CI)	Reference
Mammalian terrestrial carnivores	whole dataset	modelled	74	9.12 (2.71;30.70)	-0.66 (-1.01;-0.30)	Chapter 1
Mammalian terrestrial carnivores	small prey-feeders	modelled	40	2.64 (1.30;5.36)	0.20 (-0.20;0.61)[a]	Chapter 1
Mammalian terrestrial carnivores	large prey-feeders (single meal)	modelled	27	1.01 (0.96;1.06)	-0.33 (-0.34;-0.31)	Chapter 1
Mammalian terrestrial carnivores	large prey-feeders (complete consumption)	modelled	27	0.33 (0.19;0.58)	-0.22 (-0.40;-0.04)	Chapter 1
Mammalian terrestrial carnivores	Whole dataset	field observation	74	1.11 (0.20;6.18)	-0.48 (-0.91;-0.04)	Chapter 1
Terrestrial carnivores	/	modelled	44	28.8	-0.43[b]	Vézina (1985)
Terrestrial predators	/	modelled	49	3.00	-0.47	Peters (1983)[c]

Dataset of Chapter 1 is restricted to the order of Carnivora and focussed on vertebrate prey-feeders; dataset of Vézina (1985) is restricted to homeotherms and includes birds and mammals; dataset of Peters (1983) is restricted to homeotherms and includes birds and mammals; [a] no significant negative linear scaling for small prey-feeders; [b] M_{pred} values should be inserted in grams for the Vézina scaling formula, all other formulas are in kilograms; [c] Prey sizes used in Peters (1983) were taken from the at that time unpublished data of Vézina; large prey-feeders are divided in single meal per prey (i.e., constrained by C) or a complete consumption of the prey (i.e. over several days, assuming an absence of scavenging/kleptoparasitism).
Example for the lion (*Panthera leo*): Mpred = 175.5 kg, field KF in Meena et al. (2011) = 0.26 kills/day; KF model outcomes are: 0.30 kills/day (Chapter 1, whole); 0.18 kills/day (Chapter 1, large prey-feeder, single meal), 0.11 kills/day (Chapter 1, large prey-feeder, complete consumption), 0.09 kills/day (Chapter 1, whole, field observation), 0.16 kills/day (Vézina, 1985), 0.26 kills/day (Peters,1983).

One peculiar outlier in the large body-sized spectrum, that was not however included in the body mass scaling, is the polar bear (*Ursus maritimus*) with a bodymass of 387.5 kg, average prey weight of 4.1 kg (< C) and KF of 3.3 kills/day. As such, the polar bear can be considered a small prey-feeder. The low average prey weight stems from the combination of different small prey types seen in polar bear diet studies such as e.g. seal pups (eg. ringed seal *(Phoca hispida)*) with an average prey weight of 11 kg (Derocher et al., 2002) or Anatidae species averaged at 1.7 kg. The polar bear is known as an opportunistic forager exploiting a variety of plants and animals (Russell, 1975). Climate driven environmental changes are also forcing polar bears to adopt even more flexible foraging strategies such as prey switching, omnivory and food mixing. From a complete carnivorous lifestyle in winter (seals), polar bears switch to a more omnivorous and mixed diet on shore in summer (vegetation and geese) (Gormezano and Rockwell, 2013). Considering the winter-ecotype of the polar bear where it mainly opts for the sit-and-wait seal foraging strategy (Stirling, 1974; Gjertz and Lydersen, 1986) with an average prey weight of an adult seal of ca. 60 kg, then the model renders a KF of 0.4 kills/day which leans toward large prey-feeding; at higher kill frequencies, which appear perfectly feasible, the polar bear could be an even more selective feeder, as sometimes the species is reported as consuming only the blubber of their seal prey (Stirling and McEwan, 1975).

The real kill frequency, obtained from literature field data, scaled to carnivore mass: 1.11 [0.20;6.18] $M_{pred}^{-0.48\ [-0.91;-0.04]}$. The wide confidence interval of the scaling exponent included the KF scaling exponents for large prey-feeders (single meal strategy: $M_{pred}^{-0.33\ [-0.34;-0.31]}$; complete consumption: $M_{pred}^{-0.22\ [-0.40;-0.04]}$). The difference between scaling exponents can be due to several reasons, such as the methodology used for assessing kill frequencies in the wild. Small prey is often missed during continuous monitoring sessions in the field and might therefore underestimate the real kill frequency. However, differences might be best approached in a species-specific way: if real kill frequencies are lower than both single meal and complete consumption outcomes, the previous might be explanatory; if however the real kill frequency seems to resonate with complete consumption outcomes, the ecology of the carnivore might be explanatory (a carcass is fed on for several consecutive days which is made possible since, for instance, the leopard (*P. pardus*) moves prey into trees, caves, large burrows or dense vegetation (Sunquist and Sunquist, 2002; Balme et al., 2017) and is therefore protected from scavengers or kleptoparasites (see also 2.2.2)).

In conclusion, it seems that only the KF of large prey-feeders can be predicted by their bodymass. The central player in this part is the gut capacity (C). Since C scales to carnivore

bodymass 0.09 $M_{pred}^{1.19}$, it seems logic that the KF of large prey-feeders, that are defined by the relationship C to the average prey weight, shows a similar inverse pattern in kill frequency outcomes.

2.2.2 *The relation of gut capacity and energetic requirements: implications for kleptoparasitism and scavenging*

One major finding that stems from **Chapter 1** is the discrepancy found between the allometries of energetic requirements ($M_{pred}^{0.85}$) and gut capacity ($M_{pred}^{1.19}$). Differences in the allometric scaling of these physiological parameters, help to explain species diversification along a certain body range, which is typically done in herbivore studies (Clauss et al., 2007b; Clauss et al., 2013; Müller et al., 2013) but rather new in carnivore digestive physiology. The inequality of energetic requirements and gut capacity allometries is of a similar kind in herbivores, where gut capacity also scales higher than requirements. This implies that larger herbivores are potentially subsisting on lower quality-diets by just ingesting disproportionately more of them and they do not necessarily have to increase digestive efficiency (Clauss et al., 2013; Müller et al., 2013).

Considering this discrepancy in carnivores, this would imply that smaller sized carnivores (< 4kg) would have to eat several times a day and this to their gut fill, if they would be large prey-feeders, or several small prey until their requirements are met. This would require a fast gut clearance, i.e. short retention times that reside under a 24 hour timespan, which would not form a necessity for large body-sized carnivores. Considering the least weasel (*Mustela nivalis*) of 0.13 kg and a C < requirements, one would assume short retention times. Published retention times found in other mustelids fed whole prey average from 1.96 to 11.75 h (the Japanese marten *Mustela melampus;*Tsuji et al. (2015)) and are < 24h. A broad compilation of retention time data in carnivores together with food intake (or requirements), gut capacity and digestibility data would offer further clarity whether or not retention time can be considered as result of intake and gut capacity as is the case in herbivores (Clauss et al., 2013; Müller et al., 2013) (see also Future perspectives). One interesting challenge that should be adressed, is the broad range of body size for the domestic dog (*C. familiaris*) associated with different breeds. The effect of canine body size on gastrointestinal transit has been adressed but results are contradictory, with some authors finding an inverse relationship of gastric emptying and body size (Bourreau et al., 2004) whereas others could not find any relation (Weber et al., 2002b; Yam et al., 2004; Boillat et al., 2010b).

In contrast, larger body-sized carnivores (from ca. 4 kg upwards) do not necessarily need to consume the full amount of C in order to meet their requirements (energetic requirements < C). In theory, this would mean that these carnivores could eat in excess of their daily requirements which would further allow for larger intervals between hunting and feeding events, i.e. lower kill frequencies (the case of feast-and-famine). On the other hand, the production of food surplus enables larger body-sized carnivores to selectively (incompletely) consume carcasses (which could be in excess or meeting the energetic requirements, depending on the prey size) and it could drive other behavorial processes (e.g. hoarding, gorging and pack defence of the carcass) to safequard excess prey from kleptoparasitism and scavenging (see **Chapter 1** for species specific 'safeguard' behaviours). As such, carnivores exceeding 4 kg within the large prey-feeder functional group (iM_{prey} > C) with the assumed daily intake of C, would concern carnivores eating in excess of their requirements, explaining the lower kill frequencies found and even lower kill frequencies with guarding and complete consumption of a large prey. In conclusion, the large prey-feeding strategy appears particularly interesting for large carnivores since it could reduce their hunting energy expenditure (Carbone et al., 2007) and allow them to become 'full and lazy' (Jeschke, 2007).

2.3 Carnivore functional group dichotomy: the body size driven theory under siege

Taking into account the outcomes of the KF model (**Chapter 1**) and its allometries with M_{pred}, it is fair to say that the preassumed functional dichotomy in carnivores and its possible co-evolution with digestive physiology (bluntly stated feast-and-famine vs frequent feeding) is not a body-size-only driven feature but is of a more delicate kind: the relation of the carnivore's gut capacity versus energetic requirements together with the chosen prey size will strongly determine the functional group of the carnivore. Although the prey choice of a predator has been shown to be broadly driven by the predator size (Carbone et al., 1999), this pattern is not exclusive as shown by the study outcome in **Chapter 1**. Phylogenetic and/or ecological forces might exert similar effects on the 'choice' for a specific functional group. There is the Ethiopian wolf (*C. simensis*) for instance that although a carnivore of ca. 15 kg, with gut capacity exceeding requirements, for which hunting large prey would be energetically beneficial (Carbone et al., 2007), it is urged to hunt small rodents given its geographical location (Ethiopian highlands) (Ashenafi et al., 2005; Marino et al., 2009). Similarly, there are reports of wildcats (*F. silvestris*) hunting relatively larger rabbits instead

of small rodents (Malo et al., 2004) and African wild dogs (*L. pictus*) sustaining themselves on fairly small ungulates (Woodroffe et al., 2007). Taking it even further, species-specific reports on *what* prey a carnivore consumes (e.g. reviews of Hayward and collaborators) always report a range of prey species subdued, which, in itself, suggests different prey over time, hence adopting a different feeding strategy over time within a species. Different individuals of the same species can even be specialised in different prey (Codron et al., 2016). Also, the social status for social carnivores (Peterson and Ciucci, 2003) or the ontogeny of the carnivore (Elbroch et al., 2017) might influence the adopted strategy.

Metabolic adaptations as a result of the foraging strategy as suggested by Bosch et al. (2015), i.e. a feast-and-famine lifestyle for wolves and possibly for other feast-and-famine adherents might have led to maintaining certain enzymatic pathways to cover essential nutrient production during days without prey intake (Kreeger, 2003; Bosch et al., 2015) are therefore not necessarily a function of body size but rather the functional group as is, be it driven by ecology, phylogeny or other to be elucidated factors.

To conclude, it is urged to abandon the hypothesis put forward in this dissertation that body size would drive a whole feeding strategy, i.e. the typical assumption of a small body-sized carnivore that will consume whole prey in a frequent manner (prototype of the wild cat) and a large body-sized carnivore that will subdue large prey, only eating the highly digestible parts in an excessive way (prototype of the wolf). However, the question might be to what a carnivore is able to adapt, given certain ecological circumstances, which might be more flexible for larger carnivores.

3. Challenging domestic carnivore digestive physiology with 'ancestral' diets

Given the functional dichotomy in free-ranging carnivores studied in **Chapter 1**, there are certain assumptions on the gastrointestinal passage put forward: the group of small prey-feeders with a bodymass < 4 kg would, as a consequence of the energetic requirements and gut capacity relationship, require regular food intake hence a fast gut clearance which would not be a necessity in large prey-feeders > 4 kg. Establishing empirical relationships with datasets on gut capacity, food intake, digestibility and retention times/gastrointestinal passage would be a next step in this matter (See Future perspectives). In order to empirically study retention times in carnivores, one would have to rely on the data available across carnivore species. Passage studies in carnivores are restricted to carnivores in captivity and domestic carnivores with only small number of studies using whole prey diets (Bowland and Bowland, 1991; Leemans et al., 2015; Vásquez-Vargas and Brenes-Soto, 2015; Tsuji et al., 2015) with current knowledge on passage evidently stemming from commercial diet challenges (from dry kibble diets to canned meat diets) (e.g. Itoh et al. (1986); Peachey et al. (2000); Wyse et al. (2003); Boillat et al. (2010b)). However, it is known that intake level and the diet type strongly affect retention time outcomes and comparison between species or between different functional groups are constrained by different physical and nutritional characteristics of the diet (Hogan and Weston, 1969; Hintz et al., 1971). Hence, gathering more knowledge on the hitherto underrepresented effects of a natural diet (i.e. whole prey) on gastrointestinal physiology, more specifically gastrointestinal passage (**Chapter 2**) and faecal characteristics (**Chapter 3**), would seem to impose if one wants to study digestive physiology in an evolutionary perspective.

3.1 Whole prey diets: a matter of structure

The practice of feeding whole prey to carnivores in captivity has imposed itself in order to improve and stimulate the natural behaviour (Mellen and Shepherdson, 1997; McPhee, 2002; Hoy et al., 2010). In order to determine nutritional and energetic adequacy of whole prey, efforts have been made to assess the nutritional composition of several whole prey diets (Dierenfeld, 1993; Douglas et al., 1994; Clum et al., 1997; Dierenfeld et al., 2002; Depauw et al., 2013). Although a balanced nutrient and energy supply is of prior importance, the structure that whole prey might offer might be of equal importance. Not only do oral health

and natural behaviour benefit from the addition of structure to the diet in captive carnivores (Bond and Lindburg, 1990); recent evidence suggests a notable impact on the gastrointestinal health in captive cheetahs (*A. jubatus*) (Depauw, 2013; Whitehouse-Tedd et al., 2015). The component having a notable share in the presence of structure in a whole prey is 'animal fibre'. Animal fibre is considered the low to non-digestible (glyco)protein-rich matter present in raw bones, tendons, cartilage, skin, hair or feathers in whole prey and potential similar functions as plant-derived fibre have been suggested (Depauw et al., 2013). Additionally, it has been shown to reduce putrefactive compounds associated with protein fermentation in the hindgut (Depauw et al., 2012; 2013) and improve faecal consistency in cheetahs (Depauw et al., 2013).

The whole prey diets used in this dissertation (**Chapter 2** and **3**) consisted of day-old-chicks. Evidently, nutritional and energetic composition are strongly deviating from traditional kibble diets and even other whole prey diets (different species, different age-class) (Table 3). Since the area of focus was centred around 'structure', day-old-chicks were considered as a representative diet for whole prey including sufficient amounts of animal fibre. However, up until now, no validated quantitative nor qualitative analytical method exists to determine animal fibre.

Table 3 Component analysis and calculated energy content of whole prey diets and traditional kibble diet

	Diet		
	Chunked day-old-chicks	Dry kibble diet[c]	Whole rabbit[d]
Component (% of DM)[a]			
Dry matter (% as is)	24.9	93	31.9
Crude protein	57.3	21,3	61.0
Crude fat	22.7-26.4[b]	10.5 – 12.7[b]	26.0
Total fibrous matter[e]	38.0	28.0	/
Insoluble fibrous matter[f]	26.2	18.0	/
Crude ash	7.1	7,91	11.1
Crude fibre	2.5	1,58	1.1
TDF	/	/	3.4
Metabolisable energy (kJ/100 g DM)[g]	1672	1777	2013

[a] Unless otherwise stated; [b] Smallest value without hydrolysis, largest value with hydrolysis; [c] Pedigree ® Adult Chicken; [d] From Depauw et al. (2013); [e] Total fribrous matter according to Cools et al. (2015), this concerns animal fibre as well as plant-derived fibre; [f] Insoluble fibrous matter according to Cools et al. (2015), this concers insoluble animal fibre and plant-derived fibre; [g] The metabolisable energy is calculated by Atwater factors (16.7 × crude protein + 37.7 × crude fat + 16.7 × NfE) with NfE (Nitrogen free extract) calculated as 100 - moisture% - crude protein% - crude fat% - crude fibre% - crude ash%; DM = dry matter; TDF = total dietary fibre

When performing the commonly used techniques for plant derived fibres such as crude fibre (CF) (ISO, 1981), acid detergent fibre (ADF) (Van Soest et al., 1991) or total dietary fibre (TDF) (Prosky et al., 1985) often only small percentages of fibres are detected in animal derived products (Depauw et al., 2013). These methods were developed for plant derived materials and it is, therefore, questionable what their value is when applied to whole prey diets. Indeed, when considering the molecular structure of some potential animal fibres, these molecular structures include a significant percentage of nitrogen. Typically, TDF analysis is focused on starch digestion (addition of α-amylase and amyloglycosidase) after which a correction for total nitrogen is carried out (in order to account for undigested protein remains) (Prosky et al., 1985). As such, when applying this method to whole prey, i.e. animal fibre components, it clearly underestimates dietary animal fibre. Recently a method to quantitatively (and partly qualitative, i.e. insoluble animal fibre) estimate animal fibre has started the process of validation (Cools et al., 2015) which renders promising future perspectives in the formulation of whole prey diets. As a first, the whole prey diets used in

this dissertation (**Chapter 2** and **3**), i.e. day-old-chicks, were analyzed according to the current protocol suggested by Cools et al. (2015). Animal fibre percentages are not expressed as such, but as total and insoluble fibrous matter (Table 3). These measures include animal and plant-derived fibre (at this stage of the validation process, animal fibre-only measurements are not possible yet). Considering day-old-chicks, total fibrous matter should represent animal fibre since no plant fibre is present in this diet. However, the total fibrous matter present in the kibble diet will possibly represent a mixture of animal and plant fibre. Although results are preliminary, it becomes clear that when comparing to total fibrous matter outcomes, crude fibre analysis underestimates the amount of 'fibre' present in whole prey and even traditional kibble food. When the total fibrous matter fraction found in the day-old-chicks is compared to TDF outcomes for whole rabbit (Depauw et al., 2013), both whole prey with assumingly substantial amounts of animal fibre, the TDF analysis might underestimate fibre fractions in whole prey (Table 3). Without making any profound statements on the analytical techniques used to assess fibre in carnivorous diets, it seems that with current methods, animal fibre is underestimated. To stretch the reasoning even further, the crude protein assessed in whole prey diets that is regarded as enzymatically digestible, might even be overestimated due to the high amount of N that animal fibre contains. Additionally, the statement that whole prey diets are high in protein and low in fibre, needs to be revisited.

The faecal fermentation products, that is short-chain fatty acids (SCFA) and ammonia (NH_3), found in this study (**Chapter 3**), were comparable with fermentation products found in other canine studies using commercial kibble diets with a certain inclusion of plant fibre and commercial raw meat (Middelbos et al., 2007; Bosch et al., 2009; Beloshapka et al., 2012) and were comparable with values found in exotic felids and domestic cats fed commercial raw and cooked meat diets (Vester et al., 2008; Kerr et al., 2012) and whole prey (Depauw et al., 2013) (Table 4). The undigested parts of day-old-chicks, such as tendons, feathers and bones, might therefore have modulated the fermentative processes in the hindgut as has been shown in humans and cheetahs (Macfarlane et al., 1992; Depauw et al., 2012; 2013). However, although comparable, SCFA values found in **Chapter 3** are the lowest compared to traditional kibble diets including different plant fibre types and commercial raw or cooked beef based diets and. All of these diets contained a certain amount of plant fibre which might have caused increasing SCFA production (e.g. beet pulp (Sunvold et al., 1995)). Additionally, it is well known that small prey (e.g. day-old-chicks) have higher volume-to-surface ratios, hence are covered with more hair or feathers per unit bodymass compared to larger prey (Jethva and

Jhala, 2004). Since hairs (and feathers) are poorly fermentable and may serve as a bulking agent during fermentation (Depauw et al., 2012; 2013), it is plausible that fermentation was less in domestic dogs fed day-old-chicks. Comparing the ratios acetic acid:total SCFA; propionic acid:total SCFA and butyric acid:total SCFA of **Chapter 3** to the ratios found in an in vitro fermentation study of different animal compounds (Depauw et al., 2012), it seems that ratios are comparable to those of cartilage and glucosamine-chondroitine, which might have served as fermentation substrates in day-old-chicks (Table 4). However, the structure variation that was applied in the test diets did not seem to affect fermentative processes in the hindgut (**Chapter 3**).

Other digestive physiology parameters such as gastrointestinal passage (**Chapter 2** and **3**) might be affected as well by whole prey feeding and remained to be elucidated. Given that free-ranging carnivores can be expected to swallow chunks and pieces from different animal parts present in whole prey - which might even differ between carnivore functional groups (**Chapter 1**) and which further contributes to dietary structure - we worked with two experimental diets consisting exlusively of day-old-chicks but chunked at different die sizes (**Chapter 2**). As such, diets started off with a basal level of structure (added by animal fibre) which was further varied between diets by maintaining different 'particle sizes'.

Table 4 Average faecal dry matter (DM), faecal short-chain fatty acids (SCFA) and ammonia (NH_3) for several carnivore species and diets

Species	Test Diet/Substrate	DM (g/kg)	SCFA (mmol/kg DM)							NH3 (g/kg DM)	Ratios			Reference
			Ace	Pro	But	Val	isoBut	isoVal	Total		Ace/ SCFA	Pro/ SCFA	But/ SCFA	
Domestic dog	Chunked day-old-chicks; Fine	308.1	95.8	43.0	27.5	3.6	6.3	9.9	186.1	2.3	0.51	0.23	0.15	Chapter 3
	Chunked day-old-chicks; Coarse	296.0	99.5	39.5	26.0	3.8	5.8	9.7	184.3	2.2	0.54	0.21	0.14	
Domestic dog	Kibble diet + cellulose[a]	/	127	49	21	9.6	4.8	8.3	219.7	2.2	0.58	0.22	0.10	Middelbos et al. (2007)
Domestic dog	Low fibre kibble diet (cellulose)	379.1	140	60	30		22.1[d]		260	2.7	0.54	0.23	0.12	Bosch et al. (2009)
	High fibre kibble diet (beetpulp and inulin)	231.0	320	140	50		23.8[d]		540	3.5	0.59	0.26	0.09	
Domestic dog	Raw beef (blended)[b]	435.0	327	103.4	86.4	2.9	14.9	22.6	558.2	4.9	0.59	0.19	0.15	Beloshapka et al. (2012)
	Raw chicken (blended)[c]	437.0	343.7	123.8	75.1	2.1	11.6	17.6	573.8	4.1	0.60	0.22	0.13	
Cheetah	Whole rabbit	390	171.8	35.9	33.3	/	1.0	3.3	325.6	/	0.53	0.11	0.10	Depauw et al. (2013)
	Supplemented beef	380	202.6	73.7	47.4		6.6	13.9	255.3	/	0.79	0.29	0.19	
Cheetah	Collagen	906	5908.2	698.6	648.7	139.7	219.6	329.3	7944.1	14171.6	0.74	0.09	0.08	Depauw et al. (2012)[e]
	Cartilage	956	2924.6	1289.7	426.9	54.5	127.2	172.6	4995.5	7602.2	0.59	0.26	0.09	
	Glucosamine-chondroitine	958	2782.1	854.2	337.0	94.0	62.7	70.5	4200.6	6324.5	0.66	0.20	0.08	
Jaguar	Commercial raw beef based diet[f]	278.6	516	233	85	1.2	8.0	10.7	852.8	3.2	0.61	0.27	0.10	Vester et al. (2008)
Siberian tiger		285.8	4095	1689	532	5.2	69.9	127.0	6518	5.3	0.63	0.26	0.08	
Domestic cat	Commercial cooked beef based diet[g]	411	275.3	102.7	25.2	5.3	5.1	6.4	421.5	1.2	0.65	0.24	0.06	Kerr et al. (2012)

Ace = acetic acid; Pro = propionic acid; But = butyric acid; Val = valeric acid; isoBut = iso-butyric acid; isoVal = iso-valeric acid; Ratios = short-chain fatty acid concentration divided by the total short-chain fatty acid concentration; [a] One of 6 test diets in the study of Middelbos et al. (2007) in which several plant fibres were added to a control kibble diet; [b] ground raw beef including muscle, liver, ground beef bone, heart and premix (including plant fibres); [c] ground raw chicken including muscle, liver, bone, heart and premix (including plant fibres); [d] sum of Val, isoBut and isoVal; [e] In vitro fermentation study where several animal substrates were fermented with cheetah faecal inoculum for 72 h; [f] Nebraska Brand Special ® Beef Feline including plant fibre; [g] Raw beef based diet cooked before feeding (Central Nebraska Packing Inc.) including plant fibre

3.2 Passage of whole prey diets through the canine gastrointestinal tract

3.2.1 *Effect of dietary structure variation on passage parameters*

It is possible that small carnivores have shorter retention times compared to large carnivores as a consequence of the adopted feeding strategy (**Chapter 1**). However, before studying carnivore retention times on a broad scale, one has to know what drives gastrointestinal processes preferably on whole prey diets. In **Chapter 2**, the effect of dietary structure in whole prey on gastrointestinal passage was examined. Whole prey structure (in terms of particle size differences) did not affect transit parameters in the canine gut, i.e. a coarse chunked day-old-chick diet (die size 13 mm) did not show a significantly different transit compared to a fine chuncked day-old-chick diet (die size 7.8 mm) (**Chapter 2**). The most obvious and plausible reason should be sought in the magnitude of structure differences between diets which was only 5.2 mm and probably to small to provoke any significant difference. The reason why an addition of structure could affect gastrointestinal transit in the carnivore gut, stems from evidence in studies where structure is enhanced by adding insoluble plant fibre to traditional petfood diets. The inclusion of cellulose in a canned meat diet can decrease total transit time in dogs (Burrows et al., 1982). Sugarcane inclusion in extruded diets can, in turn, delay gastric emptying and colonic filling time in dogs (Pedreira et al., 2013). However, insoluble fibre is often ground to powder when added to a traditional kibble or a canned meat diet (e.g. alpha cellulose ® Solka Flok; Burrows et al. (1982)). Therefore, the effects on gastrointestinal passage might not originate from the structure of insoluble plant fibre and might originate from other processes. For instance, the production of SCFA is known to stimulate the secretion and production of gastrointestinal satiety hormones (Massimino et al., 1998) which can delay gastric emptying and small intestinal transit in dogs (i.e. ileal brake mechanism) (Wen et al., 1995). The latter however is associated with fermentable plant fibre and not with insoluble plant fibre such as cellulose. Additionally, Bosch et al. (2009) did not observe an increase in gastrointestinal satiety hormones in dogs when dietary fibre was increased. In contrast, variation of plant fibre particle size (coarse bran versus fine bran) is known to slow down gastric emptying in humans (Vincent et al., 1995). Although mechanisms are not completely clear, the authors suggested that the difference in gastric emptying was associated with the greater water holding capacity of coarse bran. Given

the analogies of plant fibre with animal fibre, potentially similar effects were expected although structure variation in day-old-chicks was probably too small (see above).

Comparing transit data with some other passage studies in canines on traditional diets (Table 5), transit time parameters seem to overlap, hence a preassumed structure advantage in whole prey and its possible effects on gastrointestinal passage (e.g. delaying gastric emptying) does not seem to distinctively differ from a traditional carnivore diet. However, obviously, comparing results to other trials is difficult due to different experimental designs, different canine body sizes and other differing dietary characteristics such as dietary volume and intake level (Gupta and Robinson, 1995; Lin, 1996), energy density (Wyse et al., 2001) and fat content (Meyer et al., 1994; Meyer et al., 1999) which all might influence passage through the gut.

Only one transit parameter in **Chapter 2**, differed significantly between the coarse and fine chunked prey, i.e. the maximum retention time (MaxRT; obtained with a powder marker (TiO_2)) being longer for the coarse than the fine diet. Although this concerns a very small numerical difference (33.3 h on the coarse diet versus 30.8 h on the fine diet) and therefore might not be of relevance for the question how structure affected transit, the fact that compared to the absence of a dietary difference in the total transit measure aTTT (obtained with a capsule marker) might point to a previously underestimated importance of marker choice in carnivore passage studies.

Table 5 Average transit parameters estimated with different techniques for domestic dogs fed different diets

Species	Diet	Method	Parameter	Time (hours)	Reference
Domestic dog (beagle)	Chunked day-old-chicks; fine; fed according to MER	Wireless motility capsule	GRT	15.4	Chapter 2
			SBTT	2.6	
			CTT	14.8	
			aTTT	32.8	
		TiO_2 powder marker	MRT[a]	19.5	
			MaxRT	30.8	
	Chunked day-old-chicks; coarse; fed according to MER	Wireless motility capsule	GRT	13.7	
			SBTT	2.4	
			CTT	12.2	
			aTTT	28.2	
		TiO_2 powder marker	MRT[a]	22.0	
			MaxRT	33.3	
Domestic dog (mixed breed)	225 ml radiolabelled dry kibble + 75 ml beef baby food (1772 kJ)	Wireless motility capsule	GRT[b]	8.8	Boillat et al. (2010a)
			SBTT[b]	3.5	
			CTT[b]	28.3	
			aTTT[b]	39.1	
		Scintigraphy	$T_{1/2}$-GET[b,c]	2.3	
Domestic dog (mongrel dog)	Labelled cooked egg + 1 can of meat (Alpo ® dog food; 1881 kJ)	Scintigraphy	$T_{1/2}$-GET[c]	4.8	Iwanaga et al. (2008)
			SBTT	1.7	
Domestic dog (Collie cross)	High (non-fermentable) fibre kibble diet (25 % of daily caloric intake)	Radiography with radiopaque markers (1.5 mm)	Colonic MRT[d]	12.0	Bruce et al. (1991)
		Radiography with radiopaque markers (5 mm)	Colonic MRT[d]	11.5	
Domestic dog	Canned food ; fed according to MER	Plastic beads	MRT	22.9	Rolfe et al. (2002)
		Chromium oxide powder	MRT	24.3	
Domestic dog	Whole meal bread (1 slice), skim milk (100 ml) and 5 g margarine (total energy 775 kJ)	^{13}C-octanoic acid breath test	$T_{1/2}$-GET[c]	3.58	Wyse et al. (2001)

MER = maintenance energy requirements; GRT = gastric residence time; SBTT = small bowel transit time; CTT = colonic transit time; aTTT = total transit time; MRT = mean retention time; MaxRT = maximum retention time; $T_{1/2}$-GET = gastric half-emptying time; [a] calculated according to Thielemans et al. (1978); [b] average of reported range; [c] different endpoint compared to GRT; calculated from the area under the curve of the proportion of spheres present

3.2.2 *Marker choice in carnivore passage studies: an underestimated importance*

Gastrointestinal passage studies in domestic carnivores are typically carried out in order to establish healthy individual standards which should improve the diagnosis of gastrointestinal motility disorders (Bruce et al., 1999; Washabau, 2003; Wyse et al., 2003; Boillat et al., 2010a; Boillat et al., 2010b). Gastric motility or gastric emptying has been studied extensively in dogs, not only for species specific purposes (Boillat et al., 2010a), but also to serve as an animal model for human gastric motility in physiological and pharmaceutical studies (Wyse et al., 2003).

Over the past years, numerous techniques have been developed to assess gastrointestinal passage in domestic carnivores. As reviewed by Wyse et al. (2003), techniques can be divided in diagnostic imaging techniques (radiography with the use of radiopaque liquids, meals or indigestible solids; radioscintigraphy which is considered the golden standard; ultrasonography and magnetic resonance imaging), electric resistance techniques and tracer techniques (including gastric tracers, plasma tracers and breath tracers). Clearly, these techniques require a certain amount of animal handling with repeated restrainment (Theodorakis, 1980) and even sedation (Van den Brom and Happe, 1986) which will all have its effect on gastrointestinal passage. The assessment of passage through the gastrointestinal tract would therefore benefit from methods with less invasiveness and more practicality, i.e. a simple particle marker applied to the diet of which faecal marker concentrations make it possible to assess overall digesta retention times (Thielemans et al., 1978). The latter is common practice in herbivore passage studies (Clauss et al., 2007a; Steuer et al., 2010) and has been practised in some captive carnivores (Leemans et al., 2015; Vásquez-Vargas and Brenes-Soto, 2015; Tsuji et al., 2015). However, such marker systems are only capable of assessing total tract passage without any subdivision in gastric emptying time, small bowel transit or colonic transit. Recently, wireless motility capsules that are administered orally and measure pH and temperature throughout the gastrointestinal tract that are originally designed for pharmaceutical purposes, have been used successfully to assess all elements of gastrointestinal passage in dogs (Boillat et al., 2010a). Therefore, in order to have a full gastrointestinal passage profile when dogs are fed whole prey, the IntelliCap® wireless motility capsule (Medimetrics, Personalized drug delivery group, the Netherlands) was used (**Chapter 2**). Next to the application of the capsule marker, a powder marker (TiO_2) that associates with the solid fraction of the diet (Bedford et al., 2016) was added to the test diets

as a control for total transit time. Hence, two solid marker systems were applied of different sizes: an indigestible capsule of 11 mm diameter by 26.7 mm long and an inert powder marker.

Considering the size of the capsule, it is most likely that it will not have followed the 'average' food particle through the gastrointestinal tract. It is well substantiated that once exceeding approximately 5 mm of diameter, non-food particles cannot easily pass the pylorus and leave the stomach. Instead, they are retained in the stomach until the interdigestive migratory myoelectric complex (IMMC) occurs, which propels large particles towards the duodenum (Itoh et al., 1986; Wyse et al., 2003). As such, this marker would rather represent a large 'particle' in the dietary matter that will not follow the main stream of food throughout the gastrointestinal tract but is retained in the stomach until the IMMC occurrence. This would, if whole prey is considered, relate to for instance coarse pieces of indigestible material such as bones, fur or tendons that are ingested, although it should be taken into account that large food particles might still be 'moulded' through the pylorus together with the main stream of food, in contrast to indigestible solids (Carré, 2000). The powder of TiO_2 associates with the solid mass of diets (Bedford et al., 2016), hence should have followed the whole digesta mass, and will not have been constrained by gastric retention.

The relation between total transit measures obtained by the capsule versus the powder marker seems to confirm this discrepancy between markers. The powder marker parameters that render an impression on the retention time or passage of food through the gut are the mean retention time (MRT), a measure for mean residence of the food in the gut, and the maximum retention time (MaxRT) being the timepoint at which the last digesta have left the gut (Thielemans et al., 1978). The total transit time (aTTT) is a total transit measure obtained by the capsule mimicking the transit time of the last digesta that left the stomach and is therefore similar to the MaxRT. Without subdividing data for the coarse and fine chicks, MRT values were always lower than aTTT values which clearly reflects the fact that the capsule did not reflect the passage of the 'average' food particle but rather coarse particles present in the diet. The MaxRT and aTTT are more comparable measures which was confirmed by their positive correlation (R=0.814). However, the absence of a difference in aTTT between diets compared to the presence of a difference in MaxRT between diets, points out that the TiO_2-marker was more sensitive than the wireless motility capsule. Moreover, the ratios MRT/aTTT and MaxRT/aTTT calculated in **Chapter 2** point out the importance of the relation dietary particle

size vs marker particle size: the capsule marker became a good reflection of the larger particles present in the coarse diet.

In order to study normal physiological transit on traditional kibble diets or canned meat diets, it would be preferred to use particle markers such as powder (e.g. TiO_2) given the uniformity of these diets. However, in the study of whole prey diets and their passage through the gastrointestinal gut, it is important to consider a combination of several markers reflecting different sizes of prey parts. One could therefore consider a combination of a powder marker together with different sized beads and even an addition of markers that follow the fluid and solid phase of digesta (see 3.2.3).

One important remark that should be considered of importance when studying passage through the gut is the tendency of carnivores to retain their defecations. Colonic transit times tend to show high intraindividual variability (Boillat et al., 2010a), which was also observed in **Chapter 2** and might be due to the control of defecation by the dog for reasons unrelated to digestive physiology, such as scent marking behaviour described for some canids (Parker, 2010). As such, this finding might blur our view on how passage runs through the hindgut but is in itself insuperable and might be considered as a part of natural digestive physiology of a carnivore.

3.2.3 *Do faecal characteristics on a whole prey diet point out peculiarities of passage through the canine gastrointestinal tract*

Dogs fed whole prey diets (day-old-chicks) produced two types of faeces in terms of consistency: soft, more liquid faeces alternated with firm, hard faeces (**Chapter 3**) (Fig. 3). Although this was not different between the two dietary treatments, i.e. a slight structure difference (fine vs coarse chicks) did not affect this observation, this was in contrast with the normal defecation pattern preceeding the experiment when dogs were fed a commercial dry kibble diet (Hill's Science Plan Advanced Fitness, 1570 kJ/100 g) and had more consistent faecal consistencies (personal observation). Additionally, although not quantified but rather visually observed, the firm faeces contained pronounced amounts of feathers compared to soft faeces.

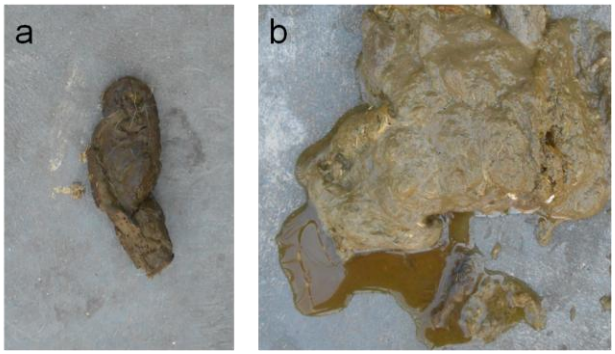

Fig. 3 Two faecal consistencies observed in dogs fed day-old-chicks
a = firm faeces; b = soft faeces

Searching the literature of carnivores fed whole prey, which was mainly restricted to feeding trials of carnivores in captivity, the observation of a faecal dichotomy has been reported for wolves (*C. lupus*) and cheetahs (*A. jubatus*) (Floyd et al., 1978; Weaver, 1993; Marker et al., 2003; Ruehe et al., 2003; Jethva and Jhala, 2004; Wachter et al., 2012). In these feeding trials wolves and cheetahs are described to produce liquid and firm faeces. Additionally, when feeding whole prey to leopards (*P. pardus*) (Lumetsberger et al., 2017), liquid faeces were sometimes produced next to the commonly collected firm faeces (Lumetsberger T., personal communication). Scanning feeding trials with carnivores fed diets such as traditional kibbles, canned meat diets, raw and cooked meat, for which faecal consistency was scored, a similar faecal dichotomy was not reported (Vester et al., 2008; Vester et al., 2010; Hooda et al., 2012; Kerr et al., 2012; Kerr et al., 2013a; Kerr et al., 2013b; Kerr et al., 2013c; Kerr et al., 2014). It is possible that the intra-individual dichotomy in faecal consistency was either not provoked by the specific diet, that it went unnoticed or unreported, that it is a species-specific finding, or that it is indeed not a common feature in carnivore digestive physiology. When looking at some faecal consistency data of feeding trials with carnivores fed commercial diets, faecal consistency variation is not noteworthy (Table 6) and might just be reflecting species effects or dietary effects.

Only one study of Hill et al. (2011) mentioned a difference in faecal moisture between defecations when dogs fed a diet enriched with texturised vegetable protein from soya. Since moisture and consistency are closely related (Zentek, 1995; Bellosa et al., 2011), as was found in this study, this might be a similar finding as found in **Chapter 3**. On the other hand, other carnivores in captivity have been challenged with whole prey (Bennett et al., 2010; Depauw et

al., 2013; Kerr et al., 2013b) as well as the domestic cat (*Felis catus*) (Kerr et al., 2014) but no faecal consistency differences were reported.

Table 6 Average faecal consistency for several carnivore species and diets

Species	N	Diet	Faecal consistency[a]	SEM[b]	Reference
Domestic dog	6	Chunked day-old-chicks[c]	3.2	1.1 (SD)	Chapter 3
Bobcat	2	Commercial raw beef based diet	3.3	0.11	Vester et al. (2008)
Jaguar	3		3.3		
Cheetah	3		3.6		
Indochinese tiger	4		3.9		
Siberian tiger	3		3.5		
Domestic dog	8	Dry kibble diet + expanded pork skin chew	2.6	0.1 (SD)	Hooda et al. (2012)
Domestic cat	9	Dry extruded diet	3.3	0.2	Kerr et al. (2012)
		Raw beef based diet	2.9		
		Cooked beef based diet	2.8		

SEM = standard error of the mean; SD = standard deviation; [a] All faecal consistencies are scored with a scale from 1 to 5 with 1 being 'hard, dry and crumbly faeces' and 5 being 'watery diarrhoea'. Half-scores were used, giving a total of 9 possible categories; [b] unless otherwise stated; [c] without subdivision in dietary treatments

The faecal consistency finding in **Chapter 3** was peculiar and deserved further attention given the similar findings for the wolf and cheetah (Floyd et al., 1978; Wachter et al., 2012). The questions of *how* and *why* this dichotomy manifests imposed themselves. By elucidating *how* it manifests, the *why* might be clarified. Observations indicate that it must be associated with structure-rich diet feeding. As discussed in 3.1, whole prey, rich in animal fibre, offers a certain degree of structure to the diet. Together wih the feeding behaviour, tearing chunks and bits from prey, a diet of varied structure or 'particle size' enters the gut. The findings from Hill et al. (2011), i.e. different moisture contents in faecal droppings from dogs fed a diet enriched with texturised vegetable protein (TVP), do not seem to abide with the 'structure' hypothesis. However, TVP typically consist of protein but also 30% of indigestible carbohydrates that may serve as fermentation substrates in the hindgut and that are suggested to cause faecal moisture differences (see below). Given the fact that a consistency difference seemingly does not occur in less structurized diets, and that firm and soft faeces seemed to differ in the

amount of animal fibre present (feathers, visual observation), a faecal dichotomy seems to associate with structure.

Several mechanisms might explain the observation of different faecal consistencies. One could bluntly state that the softer stools are just caused by infectious diarrhea. Raw meat diets can be associated with infectious agents and can impair the health of the animal (Schlesinger and Joffe, 2011). The day-old-chicks used in this dissertation were evaluated for pathogenic bacteria and the amount of Enterobacteriaceae was relatively high. However, day-old-chicks tested negative for *Salmonella* spp. and dogs remained clinically healthy througout the study. Additionally, this diarrhea would make it impossible for dogs to produce alternating firm faeces which are not indicative for diarrhea, hence suggesting that observations are of a physiological rather than pathological kind.

The liquid, runny faeces observed in wolves when fed whole prey (Floyd et al., 1978; Weaver, 1993; Peterson and Ciucci, 2003; Ruehe et al., 2003; Jethva and Jhala, 2004) have previously been associated with the ingestion of large protein-rich meals (feast meals). The digesta would pass quickly through the gastrointestinal tract, rendering osmotic imbalance, stimulation of secretion and gut motility, and inhibition of nitrogen and water absorption, which would al lead to increased water content in the faeces (Peterson and Ciucci, 2003). This could be a plausible explanation, the overload of (digestible and undigestible) protein ingestion might end up in the hindgut and cause excess protein fermentation which in its turn may cause watery faeces (osmotic imbalance) (Taylor et al., 1959; Weber et al., 2004). The first watery faeces are said to reflect the first meal of the wolves, being a large amount of muscles and organs, hence resulting in runny faeces. Afterwards, when wolves switch to the more indigestible parts of a carcass (i.e. fur, hairs), the faeces are of a more firm kind (Peterson and Ciucci, 2003). However, one important factor to consider here is that the faecal consistency dichotomy is already caused at the level of prey intake, i.e. selection and ingestion of different prey parts over time. This is in contrast with the study methodology used in **Chapter 3**, where dogs were fed chunked day-old-chicks, which caused an equal spread of prey parts in one meal. As such, the dichotomy observed is caused within the animal rather than outside the animal, and hence a separation mechanism in the gut is required. Possibly, this could mean that small-prey feeders (**Chapter 1**) will show a faecal dichotomy caused by internal separation mechanisms, whereas when observed in large prey-feeders (such as the wolf), this would be caused either at the level of ingestion as explained before, or - when not ingesting selectively - also by internal mechanisms.

It might be that the stomach plays a regulating role (see 3.2.2). As explained before, the stomach seems to regulate the passage of digesta to the duodenum with the retainement of large particles (Dressman, 1986; Itoh et al., 1986; Wyse et al., 2003; Martinez and Papich, 2009) which would lead to a separation of different sized digesta particles over time. As such it seems plausible to think that the substances such as feathers and bones of the chick diet stayed behind in the stomach and were released later on during digestion. However, studying passage of whole prey in the serval (*Felis serval*) and black backed jackal (*Canis mesomelas*), it seemed that substances such as teeth and bones were released with the first defecations (Bowland and Bowland, 1991). This would be in contrast with the assumption of a retention of large indigestible prey parts in the stomach. However, it is still possible that bolusses of indigestible prey parts are released from the stomach after which they are mixed with the content already present in the colon (Sarna, 1991), after which they are excreted first. Nonetheless, this would still require a separation at the height of the colon.

Separation mechanisms in the hindgut are common physiology in herbivores (lagomorphs, rodents and horses) (Björnhag, 1981; Björnhag et al., 1984) and some birds (Frei et al., 2015; Frei et al., 2017). The existence of these mechanisms can be explained with respect to (i) a comparative delay or acceleration of plant fibre particles to, respectively, enhance their digestion or to rid the digestive tract of them quickly (Schwarm et al., 2008); or (ii) a washing of the particulate digesta by fluid in order to direct very fine particles, including microbes, in an aborad or orad direction (Müller et al., 2011). The first principle accounts for the time-consuming process of plant fibre fermentation, retaining the easy-to-ferment small particles and excreting the large, coarse, difficult-to-digest particles quickly from the hindgut (Björnhag, 1981; Björnhag et al., 1984). In some birds, fluids and small particles can be retained in the caeca and larger particles are excreted with ordinary droppings (Björnhag, 1981; Björnhag, 1989; Frei et al., 2017). In turkey (*Meleagris gallopavo*), this mechanism has been associated with the occurrence of two faecal consistencies: solid faeces including large particles and liquid faeces including small particles (Frei et al., 2017). Given the analogies of plant fibre and animal fibre (Depauw et al., 2012; Depauw et al., 2013), i.e. recalcitrant substances such as hair, bone, feathers might compare to insoluble, coarse plant fibres (e.g. cellulose), it could be beneficial to accelerate the excretion of coarse, indigestible animal fibres from the carnivore gut. This would imply that easy-fermentable and soluble animal fibres (collagen) would reside longer in the colon. The fermentative profiles for firm and soft faeces was clearly distinct with higher indicators for protein fermentation in soft stools (i.e.

higher concentrations of SCFA and NH_3) (**Chapter 3**). Long retention in the colon of digesta can lead to high fermentation activities which in turn might lead to higher faecal scores due to an osmotic imbalance (Macfarlane et al., 1998; Macfarlane and Macfarlane, 2003; Weber et al., 2004; Hernot et al., 2005). As such, the latter seems explenatory for consistency observations: soft stools with high amounts of fermentation indicators were retained longer in the colon, and maybe even the caecum. The canine caecum harbours the highest amounts of SCFA's compared to other gut compartiments (Banta et al., 1978) and although rather small of size (Nickel et al., 1979; Abd-El-Hady et al., 2013), the caecum demonstrates some motoric activity. It generates giant migrating complexes (GMC) which may serve the expulsion of caecal content into the colon (Sarna et al., 1988).

In contrast, others state that soft stools may be a result of short digesta retention times which impair water and electrolyte absorption in the colon and potentially the absorption of SCFA (Rolfe et al., 2002), hence this would result in soft stools with high SCFA counts. Similarly, Hill et al. (2011) assigned the difference in faecal moisture over time with dogs being fed diets enriched with texturised vegetable protein, to this principle. A negative correlation of retention time with the daily numer of soft stools produced did occur (**Chapter 3**) which means more soft stools are linked with a shorter retention in the gut. However, given the fact that the powder marker used (TiO_2) associates with the solid fraction of the diets, it seems dubious to relate retention times obtained with solid markers to the frequency of soft, liquid stools. A further elaboration of passage studies in which a fluid and solid particle marker (powder and beads of different sizes) could offer more clarity in the passage of different fractions associated with whole prey through the gut (see Future Perspectives). In addition, several species with and without caecum should be studied. The ferret (*Mustela putorius*) does not possess a caecum and typically produces only hard stools on a whole prey diet (Powers and Brown, 2012; McGrosky et al., 2016).

One specific and peculiar observation in dogs that should be adressed is the lower faecal quality, in other words looser stools in large and giant dog breeds (e.g. great Dane) compared to smaller ones (Hernot et al., 2005). The authors suggested that the latter occurred due to a longer colonic residence time in larger dogs which allows for more fermentation, hence, more 'osmotic pressure' attracting more water (Weber et al., 2004). In another study, the authors suggested that the higher faecal moisture in large breeds might have to do with a higher permeability in the small intestine of large breed dogs (Weber et al., 2002a). In the light of the previously discussed faecal consistency dichotomy occuring in beagle dogs (medium size) fed

whole prey, it could be that when challenging large dog breeds with whole prey, results might be different. A faecal discrepancy could become less evident (all faeces become more moist) given their comparatively long retention times or a faecal discrepancy could still be present with a proportionally higher amount of soft stools. However, the dichotomy might still occur in large breed dogs, independently of body size, if the nature of the diet is the strongest determinant.

The question *how* the occurrence of a faecal consistency dichotomy comes about requires further investigation. As for the *why*, reasons remain highly speculative. As mentioned before, ridding the gut of the coarse indigestible compounds present in whole prey might enable carnivores to, apart from enzymatic digestion in the upper gut, efficiently use whole prey by enhancing fermentation in the hindgut (assuming the caecal hypothesis holds to be true). However, protein fermentation is also associated with the production of putrefactive compounds such as ammonia (NH_3), phenols, indoles, aliphatic amines and sulphur-rich compounds (Cummings and Macfarlane, 1991) and the presence of indigestible compounds (i.e. hairs and bones) in the hindgut might serve as a bulking agent, forming a physical barrier between substrates and bacteria and filling the large intestine, tempering protein fermentation (Depauw et al., 2013). Hence, answering the *why* seems too early at this stage, but the possibility that digesta separation may simply be a consequence of normal colon peristalsis on structured diets, without any apparent function, should not be forgotten.

3.2.4 *The dog as a carnivore species and descendant of the wolf*

Chapter 2 and **3** evaluated digestive physiology or digestive processing on whole prey diets in the domestic dog (*C. familiaris*), which was considered an example of a carnivore species, regardless of the functional group it might represent, given that the choice for a functional group is rather flexible and not physiologically fixed (**Chapter 1**). However, the choice for the domestic dog (*C. familiaris*) as a carnivore species might hold certain implications considering the pronounced feeding strategy of its ancestor, the wolf (*C.lupus*). Wolves are typically known for their feast-and-famine lifestyle. In periods of high prey abundance, wolves can subdue large prey and ingest highly nutritious animal tissues such as muscle and organs in amounts that can reach up to 22% of the wolf's own bodymass (Stahler et al., 2006; Bosch et al., 2015). After the feast meal or during periods of low prey availability, wolves can go days without catching large prey and subsist on prey leftovers (hide and bone) or opt for smaller prey in between (Peterson and Ciucci, 2003; Bosch et al., 2015). The wolf is therefore flexible and not fixed to the large prey-feast meal strategy. The latter also becomes clear when

considering the wolf throughout the world with wolves subsisting on e.g. moose in Canada's Yukon, on juvenile hares in e.g. Ellesmere Island in Canada or even salmon in Alaska (Peterson and Ciucci, 2003), or the Ethiopian wolf that lives on small prey (Ashenafi et al., 2005). One could, however, state that whenever conditions are favourable, the wolf will opt or can easily adapt to the feast-and-famine lifestyle.

The dog still shows metabolic and physiologic adaptations also present in wolves, which are considered evolutionary adaptations (e.g. downregulation of protein metabolism, extensive gastric extension) (see before). As such, the results obtained in **Chapter 2** and **Chapter 3** might be more representative for carnivores maintaining a large prey-feeding strategy and might be different in a protoype frequent-feeder such as the domestic cat (*F. catus*): if a similar experimental design would be used in cats, retention times and the absence or presence of faecal inconsistency could differ. It must be said that the experimental diets (chunked day-old-chicks) are more representative for a small prey diet in the wild (complete consumption of a whole prey). However, the main aim was to study the effect of physical structure in a whole prey diet on digestive processing in the carnivore gut. It would be interesting, in a next stage, to challenge dogs with diets mainly consisting out of meat and organs vs whole prey and register retention times and faecal consistency, and to challenge cats with different kinds of whole prey.

4. Conclusions and implications for current feeding practices in zoos and domestic carnivores

The hypothesis that carnivore functional groups in terms of feeding strategies are driven by the carnivore body size requires some adjustments (**Chapter 1**). The functional dichotomy of a feast and famine/large prey-feeding lifestyle (large prey, large highly digestible meals, low kill frequencies) versus a frequent feeding/small prey-feeding style (small prey, small meals, complete consumption of prey, high kill frequencies) in carnivores undoubtedly occurs among free-ranging carnivores. However, this is not a carnivore body-size driven feature. Rather than physiology, the concepts that drive a certain carnivore to either of these functional groups are more ecology-related and should be approached in a species specific way. The functional group of large prey-feeders however is driven by the carnivore body size, which is mainly caused by the underlying relationship of gut capacity to carnivore body size. One major finding concerning daily energetic requirements and intake capacity is their inequality, with requirements exceeding gut capacity below a 4 kg body mass threshold and gut capacity exceeding requirements below 4 kg of body mass. The latter holds several implications for on the one hand assumptions on kill frequency and gut clearance and on the other hand for ecological processes (production of carcass surplus).

Digestive physiology in carnivores on whole prey diets remains an underrepresented area of research. In order to study co-evolution of feeding strategies with the actual digestive physiology, a better understanding of the digestive physiology on natural diets (perceived as whole prey) is of the essence. **Chapter 2** and **3** tried to offer more insight in how gastrointestinal passage was affected by whole prey feeding. Gastrointestinal passage in carnivores fed whole prey should be studied carefully with a marker spectrum of different particle sizes. Additionaly, the preassumed monophasic movement of whole prey through the gut is countered and indications on a possible separation mechanism in the gut of carnivores emerged.

As findings from **Chapter 1** clearly contribute to carnivore ecosystem knowledge, this dissertation offers new insights for carnivore nutrition (domestic and captivity) in a comparative way. This dissertation did not try to offer specific guidelines for petfood formulations or diets for carnivores in captivity; instead, it tried to broaden the framework of feeding practices and management in a comparative way in order to shed new light on current nutritional disorders. For instance, the wildcat (*F. silvestris*) is a small carnivore that typically

opts for a small prey-feeding strategy, hence requiring several kills per day (MacDonald et al., 1984; Bradshaw, 2006). Nowadays, domestic cats or wild cats kept in zoos are often offered single meals per day, which partly contributes to the increasing problem of obesity in cats (Laflamme, 2006; Bissot et al., 2010; Deng et al., 2013). New strategies adopted to overcome the obesity or to manage weight in domestic cats, include the practice of increasing feeding frequency (Deng et al., 2013). Lions (*P. leo*) in captivity often suffer from problems associated with dietary over-supply such as obesity, inactivity and stereotypy. By adjusting their feeding management from daily food provision to the inclusion of carcass guarding and fasting days, which relates to their natural feast and famine lifestyle, the food digestibility and body weight might be improved (Altman et al., 2005).

The practice of carcass feeding to carnivores in zoos has gained increasing attention for its beneficial effects on natural behaviour, oral and gastrointestinal health (Bond and Lindburg, 1990; Mellen and Shepherdson, 1997; McPhee, 2002; Hoy et al., 2010; Depauw et al., 2013; Whitehouse-Tedd et al., 2015). Futher elaboration on the effects of whole prey feeding on digestive physiology is therefore of the essence to establish balanced feeding practices. Recently, the practice of feeding raw meat diets such as BARF foods (bone and raw food) and whole prey has gained more popularity in domestic carnivores as well. However, raw meat diets are often associated with diarrhea (Schlesinger and Joffe, 2011) which might not be as straightforward as thought before, since (alternating) liquid faeces might be a physiological response to a raw meat diet (if infectious diarrhea is excluded) (**Chapter 3**). Research concerning whole prey feeding is therefore imposing to unravel possible advantages and disadvantages where a knowledge on the whole prey associated digestive physiology is crucial.

In conclusion, when encountered with a certain carnivore, one could rely on the kill frequency model built in this dissertation to learn more about its feeding strategy in the wild with certain assumptions on the species specific ecology. Whole prey feeding requires further attention in terms of digestive physiology in order to establish balanced feeding management practices for domestic carnivores and carnivores in captivity.

5. Future perspectives

Future research on allometries between carnivore body size and digestive physiology parameters together with digestive physiology studies on whole prey are imposing and can be summarised in the following points:

Empirical datasets on gut capacity, food intake, retention times and additionally digestibility in carnivores have not been combined and analyzed so far, although common practice in herbivores. Findings from **Chapter 1** encourage the consideration of the allometries with body size of each parameter in order to explain species diversification in terms of physiology.

Gastrointestinal passage or retention times should be further studied on whole prey diets. Not only would this benefit the empirical dataset mentioned above, it would further elucidate gastrointestinal gut mechanics:

As a first, it should be clarified whether faecal consistency differences are indeed present when feeding whole prey, and if this is really linked to strucure-rich diets. Therefore, carnivores should be challenged with commercially available diets such as canned meat (low in structure) and a whole prey diet (rich in structure). Prey intake should be carefully monitored in order to distinguish external separation, i.e. selective feeding behaviour.

Second, several carnivore species should be studied, including domestic carnivores and carnivores in captivity. The choice for a specific species should be based on the presence of a caecum. For instance, the wolf (*C. lupus*), lynx (*Lynx lynx*), domestic dog (*C. familiaris*) and domestic cat (*Felis catus*) which all possess a caecum should be compared to carnivores without a caecum such as their are the wolverine (*Gulo gulo*), brown bear (*U. arctos*) and ferret (*M. putorius*). The latter should further clarify whether or not the caecum is involved in separating dietary fractions of whole prey and whether this should be species specific.

Passage markers that follow different digesta fractions should be added to experimental diets: one for the solid phase (mordanted hay particles, physically similar to hair), and one for the fluid phase (Co-EDTA) (Gull et al., 2015). The fluid marker should, under the conditions of an active separation mechanism, be enriched in the soft faeces, as shown in rabbits or poultry (Franz et al., 2011; Frei et al., 2017). In addition, larger sized beads could be added to experimental diets in order to mimick large pieces ingested from whole prey.

Finally, faecal patterns and characteristics (such as microbiome analysis, protein content as well as animal fibre content) should further clarify separation mechanics in the gut.

To conclude, further dissecting the functional dichotomy in carnivore feeding strategy will help to better understand carnivore species diversification. Elucidating the role of body size and the whether or not associated physiological and metabolic adaptations can broaden the framework of current feeding practices in domestic carnivores and carnivores in captivity. Additionally, the digestive physiology associated with whole prey feeding should be further clarified.

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Summary

Mammalian carnivores are highly diverse in numerous aspects of their morphology, behaviour and ecology. Similarly, great variety exists among carnivore dietary habits. Carnivores are often thought of as just 'meat eaters' with a simple digestive tract; however the opposite holds true. Carnivore species range from completely herbivorous (e.g. the giant panda, *Ailuropoda melanoleuca*) to omnivorous (e.g. brown bear, *Ursus arctos*), insectivorous (aardwolf, *Proteles cristatus)* and even frugivorous (e.g. kinkajou, *Potos flavus).* Considering carnivore digestive physiology, there is no simple prototype, and diversity occurs within gastrointestinal morphometry, allometry and transit as well as metabolic pathways. Understanding from where digestive physiology has evolved can broaden our views on current nutritional practices and broaden the framework on how to approach nutritional disorders (such as obesity) in domestic carnivores and wild carnivores in captivity. Recently, the suggestion was made that diversity seen in terms of physiology might be driven by feeding strategies in the wild, or better has co-evolved with strategies maintained by free-ranging carnivores. Hence, a better understanding of carnivore feeding strategies is required. With a focus on vertebrate feeders, it seems that a functional dichotomy exists in terms of feeding strategies among carnivores. Reports in the literature describe large carnivores hunting large prey, subsequently ingesting large amounts of highly digestible prey body parts and this at low kill frequencies (i.e. feast and famine lifestyle or large prey-feeding). Small carnivores though subdue small prey, consume it entirely and hunt frequently (several times per day) (i.e. frequent feeding lifestyle or small prey-feeder). With this concept, it seems that feeding strategies are driven by carnivore body size. There is the well described pattern in literature that carnivores switch from small to large prey-feeding at a body mass threshold of approximately 10-20 kg. As such, it is possible that carnivore body size indeed dictates the choice for a certain prey size, hence dictates a whole feeding strategy.

In **Chapter 1**, the feature carnivore kill frequency was modelled and scaled to carnivore body size. Kill frequency was considered an important part of a carnivore feeding strategy and its scaling to body mass could render important insights concerning the body size driven theory. The kill frequency model took into account carnivore mass, prey mass, carnivore specific maintenance energy requirements and metabolisable energy in prey, hunting pack size, selective feeding and carnivore gut capacity. Carnivores were subdivided in two functional groups based on the relationship gut capacity (C) and pack corrected prey mass (iMprey). Gut capacity and its allometries to body size have been acknowledged an important physiological character that helps to explain species diversification in herbivores, but has received little

attention in carnivore physiology and allometries with body size. Gut capacity was a central player in the appointment of a certain carnivore to a functional group. If iMprey exceeded C, than the carnivore was considered a large prey-feeder. If C exceeded iMprey than the carnivore was considered a small prey-feeder. In general carnivore kill frequency scaled negatively to carnivore body size with a scaling exponent of -0.66. When considering carnivore functional groups, a negative relationship with body size was present for large prey-feeders but absent for small prey-feeders. Although the large prey-feeding strategy occurs at any carnivore size, it is more frequent among larger species. Small prey-feeders are more variable in kill frequency outcomes and do not show a body size scaling, and also occur with the whole body size spectrum. Comparing energetic requirements of carnivores to their gut capacity, small carnivores would have to eat several times per day to their full gut capacity in order to meet their daily energy requirements, hence requiring fast digestion and gut clearance. This would not be required for large carnivores. They do not have to eat to their full gut capacity or do not have to eat every day which can reduce kill frequencies, or drives other ecological processes such as kleptoparasitism, scavenging or selective (incomplete) carcass consumption. Although carnivore feeding strategies are not completely driven by body size, it seems particularly interesting for large carnivores to opt for a large feeding strategy since it can allow them to get 'full and lazy' and reduce hunting efforts.

Carnivore energetic requirements and gut capacity relate differently for different sized carnivores. From a threshold of about 4 kg carnivore body mass, gut capacity goes from being lower than daily energetic requirements to exceeding requirements (**Chapter 1**). This implies several hunting events per day for small carnivores with a fast gut clearance which would be necessary for larger carnivores. Hence, the digestive physiology parameter of gut clearance or retention time might be a feature that is driven by body size hence required further investigation. Digesta retention times in carnivores are typically studied in domestic carnivores or carnivores in captivity on commercial diets such as traditional kibbles and canned meats with a broad spectrum of technologies. However, in order to study retention time and its possible co-evolution with body size, studies on whole prey diets are preferred with technologies that prove to be least invasive and practical (perceived by us as a simple marker added to the diet, of which faecal concentrations over time help to establish gastrointestinal retention time). As a first step, we wanted to challenge the dog, as a carnivore species with whole prey in order to see what drives gastrointestinal passage in carnivores (**Chapter 2** and **3**). Feeding whole prey implies feeding of 'animal fibre'. Animal fibre is

considered the low to non-digestible (glyco)protein-rich matter, such as raw bones, tendons, cartilage, skin, hair or feathers present in whole prey and has been shown to share analogies with plant-derived fibres. In **Chapter 2**, domestic dogs (*Canis familaris*) were fed day-old-chicks (whole prey-like) of two different particle sizes. Thus, the structure that animal fibre automatically provides was varied by a dietary particle size difference. Gastrointestinal passage was measured with two marker systems, a powder applied to the diet and a wireless motility capsule that measures pH and temperature in the gut, hence provides information on gastrointestinal passage. Transit parameters were not affected by dietary structure although this would mainly be caused by the magnitude of the particle size difference between the two diets, which was too small. However, one major conclusion from **Chapter 2** is that marker choice is important and in order to follow different sized dietary particles, different sized marker particles are necessary.

When studying faecal characteristics of dogs fed day-old-chicks varied in particle size (**Chapter 3**), it seemed that a faecal consistency dichotomy occurred. Soft versus hard faeces were observed intra-individually in an almost alternating pattern, and this for both diets (i.e. no effect dietary treatment). The occurrence of two faecal types in terms of consistency has also been observed for carnivores in captivity (wolf, *Canis lupus* and cheetah, *Acinonyx jubatus*), suggesting a mechanism in the gastrointestinal tract that causes a dichotomy in faecal consistency which might be linked to whole prey feeding (i.e. structure-rich diets). Differences in faecal consistency is usually not observed on traditional kibble diets. The fermentative profiles of both faecal types differed, with higher protein fermentation parameters found in soft stools. Several mechanisms are put forward in this dissertation in order to explain the observed dichotomy. There could be a regulating role of the stomach that could retain the large, coarse particles, since it is known for non-food solids that once exceeding 5 mm of diameter, these solids cannot pass the gastric pylorus. Hence, coarse particles such as feathers and bones could be retained in the stomach, which might have caused separation of different sized digesta particles which in turn might have led to the faecal dichotomy. However, one contradiction is the fact that large and coarse particles such as teeth and bones present in whole prey are known to be expulsed priorly from the gut in the serval (*Felis serval*) and black backed jackal (*Canis mesomelas*). Similar separation mechanisms as well known in herbivores might be operating in the carnivore hindgut. In mammalian herbivore hindgut fermenters, separation mechanisms often manifest in order to expulse difficult-to-ferment, coarse particles and to retain smaller easy-to-ferment particles and in this

way, account for the time-consuming process that plant fibre fermentation holds. When considering the faecal dichotomy in a comparative way, it is possible that the more easy-to-ferment, small particles (e.g. collagens) present in whole prey were retained longer in the colon, or even caecum and the recalcitrant, difficult-to-ferment fractions (hears, feathers, bones) were expulsed more quickly from the gut. However, further research is required to unravel underlying gut mechanics causing a faecal consistency dichotomy. Although still precarious, it seems that whole prey provokes certain effects on gastrointestinal transit in carnivores, and this requires further investigation in order to establish norms of digestive physiology on whole prey diets which would be necessary if one wants to consider the allometries of digestive physiology with carnivore body size.

In conclusion, the large prey-feeding strategy is particularly interesting for large carnivores because it enables them to reduce hunting efforts. Overall, the choice for a specific feeding strategy is rather ecologically driven than body size driven. Gastrointestinal passage, as a digestive physiology parameter, on whole prey diets requires further attention in order to establish digestive physiology norms.

Samenvatting

Soorten die behoren tot de orde der Carnivora binnen de klasse der Mammalia, vertonen een enorme diversiteit. Niet alleen blijkt het gedrag, de morfologie of de ecologie heel divers, ook de voedingsgewoonten vertonen veel verschillen. Wanneer het begrip 'carnivoor' wordt aanschouwd, wordt dit vaak geassocieerd met echte vleeseters die een simpel maagdarmstelsel hebben. Echter, carnivoren kunnen leven van een compleet plantaardig dieet (bv. de grote panda, *Ailuropoda melanoleuca)* tot een omnivoor dieet (bv. de bruine beer, *Ursus arctos*), een insectivoor dieet (bv. de aardwolf, *Proteles cristatus*) en zelfs van een frugivoor dieet (bv. de kinkajou, *Potos flavus*). De verteringsfysiologie toont ook menig verschil. Zo kunnen de intestinale morfometrie, allometrie en transit alsook metabole processen verschillen binnen de orde der Carnivora. Door beter te begrijpen hoe deze verteringsverschillen zijn ontstaan of waaruit ze zijn geëvolueerd, kan de verteringsfysiologie van carnivoren ons helpen het huidige voedingsmanagement van gedomesticeerde carnivoren en carnivoren in gevangenschap te verbeteren alsook onze blik te verruimen bij de studie naar voedingsgerelateerde stoornissen. Het is pas sinds kort dat een mogelijke verklaring voor de diversiteit in carnivore verteringsfysiologie werd gezocht in de voedingsstrategieën van wilde carnivoren. Het bestuderen van deze voedingsstrategieën is daarom relevant. Uit de literatuur blijkt een functionele dichotomie in carnivore voederstrategieën te bestaan. Enerzijds vindt men grote carnivoren die jagen op grote prooien, waarna zij overvloedige maaltijden consumeren met enkel de meest verteerbare delen van de prooi, en dit aan een lage frequentie (overvloed-en-honger strategie). Anderzijds zijn er de kleine carnivoren die typisch kleine prooien vangen, deze volledig consumeren en dit aan een heel hoge frequentie (frequente voedingsstrategie). De gegevens lijken aan te geven dat de voedingsstrategie waarvoor een carnivoor opteert, gedreven wordt door zijn eigen massa: vanaf 10-20 kg eigen lichaamsgewicht zal de carnivoor overgaan van een kleine prooi naar een grote prooi. Het is dus mogelijk dat de massa van de carnivoor een complete voedingsstrategie bepaalt.

In **Hoofdstuk 1** werd een model gebouwd om de 'jaagfrequentie' of beter 'maaltijdfrequentie' in te schatten en deze werd vervolgens gerelateerd aan de massa van de carnivoor. De maaltijdfrequentie is een belangrijk deel van een voedingsstrategie en de relatie ervan tot de massa van de carnivoor kan ons meer inzicht verschaffen over hoe deze massa een voedingsstrategie heeft bepaald. Het model dat werd ontwikkeld in deze thesis is uniek gezien verschillende belangrijke aspecten voor het eerst in acht werden genomen, namelijk behalve het gewicht van de carnivoor en zijn prooi, ook de specifieke energiebehoeften van de carnivoor en de metaboliseerbare energie aanwezig in de prooi, de grootte van de roedel

indien het een sociaal species betrof, aanpassingen omtrent selectief consumeren, en als laatste de maagcapaciteit. Carnivoren werden onderverdeeld in twee functionele groepen: als de maagcapaciteit de prooimassa (gecorrigeerd voor de roedelgrootte) overschreed, dan werd de carnivoor een frequente eter geacht en wanneer de prooimassa de maagcapaciteit overschreed, dan werd de carnivoor ingedeeld bij de overvloed-en-honger groep. Over het algemeen was de maaltijdfrequentie negatief gerelateerd aan de massa van de carnivoor met een negatieve exponent van -0.66. Wanneer we beide functionele groepen beschouwen vinden we deze negatieve relatie ook terug bij de overvloed-en-honger groep, echter, deze relatie werd niet teruggevonden bij de frequente-eter-groep. Het jagen van een grote prooi met het daarbij consumeren van goed verteerbare delen (zoals spieren en organen) wordt gedreven door de massa van de carnivoor. Deze strategie komt frequenter voor bij grote carnivoren maar komt voor over het volledige gewichtstraject van carnivoren. Carnivoren die voor een frequente consumptiestrategie opteren, zijn meer variabel en komen ook voor over het volledige massa spectrum. Wanneer we de dagelijkse energiebehoeften vergelijken met de maagcapaciteit van carnivoren, blijkt het dat kleine carnivoren meerdere keren per dag dienen te eten (en dus jagen) om aan hun dagelijkse energiebehoeften te voldoen. Dit vereist bijgevolg een snelle vertering en maagdarmlediging. Grote carnivoren dienen dit niet te doen, zij hoeven hun maag niet vol te eten, maar kunnen zich veroorloven om bijvoorbeeld elke andere dag te eten wat ervoor zorgt dat hun maaltijdfrequenties lager komen te liggen. Dit kan er ook voor zorgen, wanneer zij een grote prooi jagen, dat er een karkasoverschot ontstaat wat op zich andere ecologisch processen kan sturen zoals kleptoparasitisme, het schooien van prooi of het incompleet consumeren van de enkel goed verteerbare karkasdelen. Ook al worden de carnivore voedingsstrategieën niet compleet door de carnivore massa gedetermineerd, het lijkt toch interessant voor grote carnivoren om voor de overvloed-en-honger strategie te opteren gezien het hen toelaat zich vol te eten en 'lui' te zijn.

Zoals besproken verhouden de energiebehoeften en maagcapaciteit zich anders voor verschillende carnivoormassa’s. Onder een drempelwaarde van 4 kg carnivore massa liggen de energiebehoeften boven de maagcapaciteit terwijl deze onder de maagcapaciteit liggen boven deze 4 kg (**Hoofdstuk 1**). Dit betekent dat kleine carnivoren meerdere keren per dag dienen te jagen en eten, wat een snelle vertering en maagdarmlediging vereist. Dit is niet noodzakelijk voor grote carnivoren en daarom lijkt het dat de maagdarmtransit ook gedreven kan worden door de grootte van de carnivoor. Een duidelijke analyse van wat nu de maagdarmpassage van een carnivoor drijft, bleek zich op te dringen. De maagdarmtransit van

carnivoren wordt vaak bestudeerd in gedomesticeerde carnivoren en carnivoren in gevangenschap wanneer zij commerciële diëten worden gevoederd (droge korrels en blikvoeder). Echter, wil men de co-evolutie van maagdarmpassage met carnivore massa bestuderen, dan lijkt het logischer om deze carnivoren te voederen met een volledig prooi-dieet om dichter bij hun natuur te blijven. Ook al zijn technieken om passage in te schatten overvloedig voorhanden, lijkt een simpele techniek zoals het toevoegen van een merker aan het dieet en het analyseren van merkerconcentraties in de faeces (wat een idee geeft over de maagdarmpassage) de meest betrouwbare techniek. Daarom werd getracht maagdarmpassage te bestuderen in de gedomesticeerde hond (*Canis familiaris*) wanneer deze een volledig prooi-dieet werd gevoederd (**Hoofdstuk 2** en **3**). Door het voederen van volledige prooi, wordt een substantiële hoeveelheid dierlijke vezel toegevoegd aan het dieet. Dierlijke vezel wordt beschouwd als de eiwitrijke delen van een prooi die enzymatisch slecht verteerbaar zijn zoals beenderen, pezen, kraakbeen, huid, haren of veren. In **Hoofdstuk 2** werden gedomesticeerde honden gevoederd met eendagskuikens. Deze eendagskuikens werden gehakt op verschillende partikelgroottes: fijn gehakt en grof gehakt. Op die manier werd de structuurwaarde van het dieet, dat automatisch aanwezig is wanneer men volledige prooi voedert, gevarieerd. De maagdarmtransit werd gemeten met twee merkersystemen, enerzijds een poeder dat werd vermengd met het dieet en anderzijds een draadloze motiliteitscapsule die de pH en temperatuur meet doorheen het maagdarmstelsel en bijgevolg een indruk geeft van de maagdarmtransit van de honden. De maagdarmpassage werd niet beïnvloed door het dieet wat impliceert dat het verschil in structuur in deze studie geen effect had op maagdarmtransit. Echter, dit kwam hoogstwaarschijnlijk door een te klein structuurverschil tussen beide diëten. De voornaamste conclusie uit **Hoofdstuk 2** is het belang van de merkergrootte. Een dieet dat van nature varieert in partikelgroottes zoals volledige prooi of een dieet dat artificieel gemodificeerd werd in termen van partikelgrootte, vereist passagemerkers die elk van deze fracties reflecteert.

De faecale consistentie van honden die een volledige-prooi-dieet werden gevoederd (met ook hier een partikelgrootte verschil) (**Hoofdstuk 3**), bleek te alterneren binnen elke hond: zachte, vloeibare faeces werden afgewisseld met harde faeces. Dergelijke faecale dichotomie werd ook vastgesteld bij enkele wilde carnivoren in gevangenschap die ook volledige prooi werden gevoederd (de wolf (*Canis lupus*) en de cheetah (*Acinonyx jubatus*)). Uit deze observaties veronderstelden wij een tot nu toe ongekend mechanisme in het maagdarmstelsel dat deze dichotomie veroorzaakt en dat mogelijks gekoppeld is aan het voederen van een structuur-

rijke volledige prooi. Ook hier werd geen verschil gezien tussen beide diëten, maar wel wanneer het volledige- prooi-dieet werd vergeleken met het traditionele korreldieet gevoederd voor de aanvang van het experiment (dit werd echter niet gekwantificeerd). Het fermentatieprofiel van beide soorten faeces toonde een hogere concentratie van eiwitfermentatie-indicatoren in de zachte faeces vergeleken met de harde faeces. Deze vaststelling leidde tot verschillende hypothesen om deze dichotomie te verklaren. Vooreerst werd een regulerende rol van de maag beschouwd. Het is geweten dat partikels van meer dan 5 mm diameter worden weerhouden in de maag en de pylorus niet meteen kunnen passeren met de rest van de digesta. Het zou daarom kunnen dat de grote, grove partikels van het volledige-prooi-dieet achterbleven in de maag wat een scheiding van voedingspartikels veroorzaakt zou kunnen hebben, en daardoor een faecale dichotomie. Op een hele-prooi-dieet scheiden de serval (*Felis serval*) en de zadeljakhals (*Canis mesomelas*) echter eerst de grove partikels zoals tanden en beenderen uit, wat die hypothese niet ondersteunt. Vanuit breder perspectief vinden we een andere mogelijke verklaring bij herbivore species. Sommige herbivore zoogdieren en vogels vertonen separatiemechanismen ter hoogte van het colon en caecum die het mogelijk maken grove, slecht fermenteerbare partikels snel te verwijderen van het maagdarmstelsel en kleine, goed fermenteerbare partikels langer bij te houden in het maagdarmstelsel om tegemoet te komen aan het tijdrovende gegeven van plantvezelfermentatie. Wanneer de verschillende fracties van dierlijke vezel worden beschouwd, is het dus mogelijk dat de beter fermenteerbare fracties (bv. collageen) langer worden bijgehouden in het colon of caecum en de grove, slecht fermenteerbare fracties (zoals haren, beenderen, veren) snel worden verwijderd van het maagdarmstelsel. Verder onderzoek is nodig om de onderliggende mechanismen die een faecale dichotomie veroorzaken te ontrafelen. Maagdarmpassage blijkt in elk geval sterk te worden beïnvloed door het voederen van volledige prooi. Dit verdient verder onderzoek, wil men maagdarmpassage relateren aan de massa van carnivoren.

Als conclusie kan men stellen dat de voedingsstrategie 'overvloed-en-honger' wordt gedreven door de massa van carnivoren wat relevant is voor grote carnivoren omdat zij op die manier hun energie nodig voor jagen kunnen verlagen. De keuze voor een bepaalde voedingsstrategie blijkt echter sterker gedreven door ecologie dan fysiologie. Maagdarmpassage, als een belangrijk deel van de verteringsfysiologie, moet verder worden bestudeerd met volledige prooien om de wetmatigheden in de verteringfysiologie van carnivoren te begrijpen.

Appendices

Appendix 1 List of carnivore families, species, average carnivore mass and average prey weight of most commonly subdued prey included in the dataset for KF modelling.

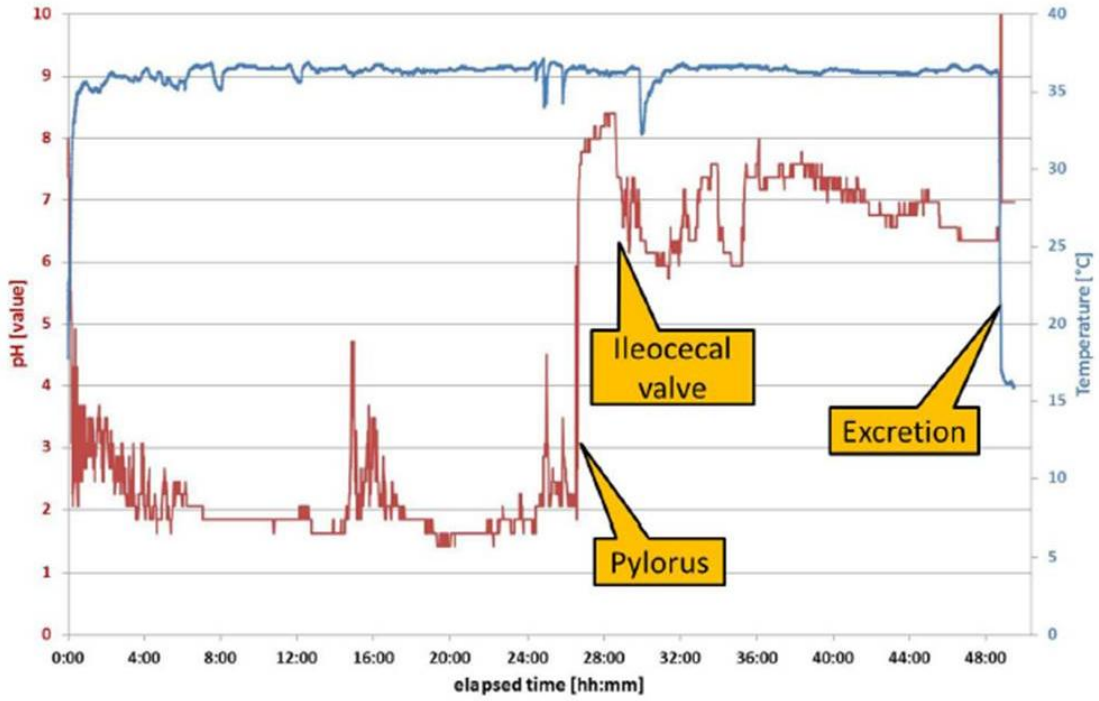
Carnivore family	**Carnivore species**	**Carnivore mass**	**Prey mass**
word	***word***	***kg***	***kg***
Viverridae	Viverra tangalunga (Malay civet)	8.0	0.001
Canidae	Lycalopex/Dusicyon vetulus (hoary fox)	3.4	0.001
Canidae	Pseudalopex/Lycalopex fulvipes (Darwin's fox)	8.5	0.004
Canidae	Vulpes bengalensis (Indian fox/Bengal fox)	2.4	0.001
Herpestidae	Herpestes auropunctatus/javanicus (small Indian/Asian mongoose)	2.3	0.001
Herpestidae	Herpestes naso (long nosed mongoose)	2.3	0.001
Canidae	Vulpes rueppellii (sand fox)	2.3	0.001
Canidae	Canis simensis (Ethiopian wolf or Simien jackal)	15.0	0.190
Canidae	Lycalopex/Pseudalopex griseus (grey fox)	4.9	0.010
Canidae	Chrysocyon brachyurus (maned wolf)	23.0	0.073
Felidae	Felis chaus (jungle cat)	10.0	0.076
Canidae	Canis aureus (golden/Asian jackal)	11.0	0.229
Herpestidae	Atilax paludinosus (water mongoose)	3.3	0.027
Felidae	Felis/Leopardus wiedii (margay)	3.3	0.029
Felidae	Felis nigripes (black-footed cat)	2.1	0.024
Mustelidae	Mellivora capensis (honey badger, ratel)	10.0	0.189
Herpestidae	Viverricula indica (lesser oriental civet)	3.0	0.051
Mustelidae	Martes flavigula (yellow throated marten)	2.5	0.100
Viverridae	Paguma larvata (Himalayan palm civet)	4.3	0.100
Herpestidae	Herpestes urva (crab-eating mongoose)	2.3	0.051
Canidae	Vulpes vulpes (red fox)	8.3	0.234
Canidae	Vulpes chama (cape fox)	4.0	0.100
Felidae	Felis/Catopuma temmincki (Asian golden cat)	11.5	0.345
Canidae	Canis rufus (red wolf)	30.0	2.860
Felidae	Puma/Felis yagouaroundi (jaguarundi)	6.8	0.222
Felidae	Felis/Leopardus tigrina (little spotted cat)	2.3	0.062
Canidae	Vulpes ferrilata (Tibetan sand fox)	7.0	0.263
Eupleridae	Cryptoprocta ferox (fossa)	12.0	0.504
Viverridae	Genetta genetta (common genet)	2.0	0.072
Canidae	Speothos venaticus (bush dog)	6.0	2.875

Felidae	Prionailurus/Felis bengalensis (leopard cat)	5.0	0.227
Mustelidae	Taxidea taxus (american badger)	8.0	0.417
Mustelidae	Mustela eversmanni (steppe polecat)	1.7	0.094
Canidae	Vulpes velox (swift fox)	2.4	0.145
Canidae	Vulpes macrotis (kit fox)	1.7	0.108
Felidae	Felis/Leopardus pardalis (ocelot)	13.6	1.269
Felidae	Felis/Otocolobus manul (Pallas's cat)	3.5	0.263
Felidae	Felis catus (feral cat)	3.9	0.311
Felidae	Felis/Prionailurus iriomotensis (iriomote cat)	3.7	0.300
Mustelidae	Martes foina (beech or stone marten)	1.7	0.128
Felidae	Felis/Leopardus geoffroyi (Geoffroy's cat)	4.0	0.352
Canidae	Alopex lagopus (arctic fox)	5.2	0.594
Mustelidae	Galictis cuja (lesser grison)	2.0	0.189
Mustelidae	Martes americana (American marter)	0.7	0.060
Mustelidae	Martes martes (European pine marten)	1.3	0.126
Canidae	Lycalopex/ Pseudalopex culpaeus (culpeo fox)	8.7	1.188
Felidae	Felis/Lynx pardina/pardinus (Spanish/Iberian lynx)	11.5	1.800
Mustelidae	Mustela putorius (European polecat)	0.8	0.076
Felidae	Felis sylvestris (African wild cat)	5.5	0.780
Canidae	Lycaon pictus (African wild dog)	26.5	49.635
Felidae	Felis/Leopardus colocolo (Pampas cat)	3.0	0.400
Felidae	Panthera onca (jaguar)	90.0	25.873
Felidae	Panthera leo (lion)	175.5	233.395
Canidae	Canis lupus (gray wolf)	43.2	95.574
Viverridae	Viverra civetta (African civet)	8.0	2.140
Canidae	Canis latrans (coyote)	15.8	17.202
Mustelidae	Mustela vison (American mink)	1.2	0.284
Felidae	Felis canadensis (Canadian lynx)	11.2	4.180
Felidae	Puma/Felis concolor (puma)	67.6	43.435
Canidae	Canis mesomelas (black-backed jackal)	9.8	23.550
Felidae	Felis rufus (bobcat)	9.7	5.375
Felidae	Felis/Caracal caracal (caracal)	12.5	8.436
Felidae	Panthera tigris (tiger)	156.3	163.300
Mustelidae	Mustela lutreola (European mink)	0.6	0.256
Hyenidae	Crocuta crocuta (spotted hyena)	63.0	140.929

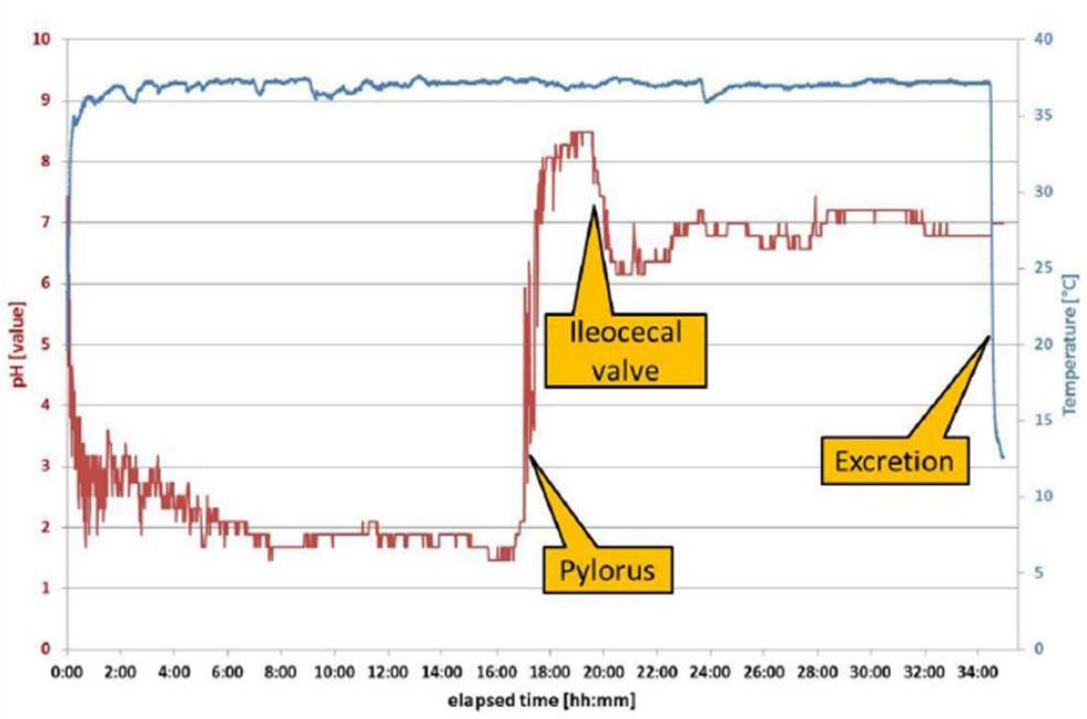
Felidae	Acinonyx jubatus (cheetah)	46.5	47.278
Mustelidae	Gulo gulo (wolverine)	19.5	18.722
Mustelidae	Mustela nivalis (least weasel)	0.1	0.061
Canidae	Cuon alpinus (dhole)	15.8	154.341
Felidae	Panthera uncia (snow leopard)	43.8	75.455
Felidae	Felis lynx (Eurasian lynx)	20.1	32.079
Herpestidae	Herpestes ichneumon (Egyptian mongoose)	2.3	2.985
Felidae	Panthera pardus (leopard)	53.8	134.700
Mustelidae	Mustela erminea (ermine, stoat)	0.2	0.763

Appendix 2 pH and temperature profile of the gastrointestinal tract obtained with a wireless motility capsule per dog for both dietary treatments (fine (7.8 mm) and coarse (13 mm) chunked day-old-chicks).

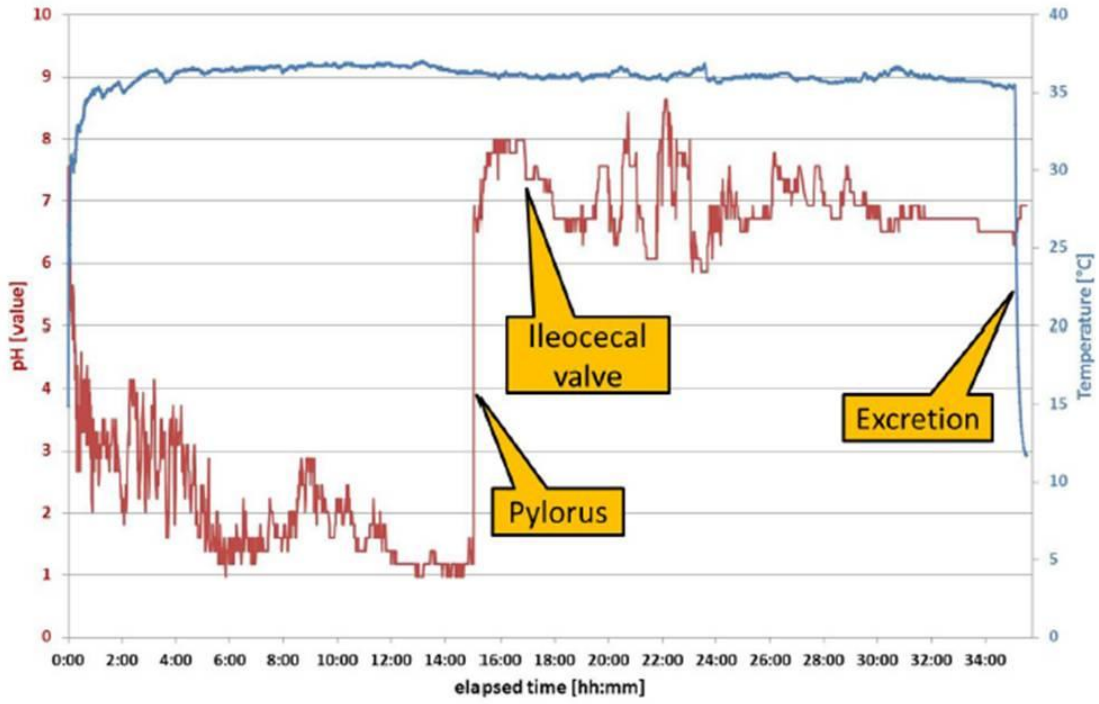
Dog 1 Fine diet



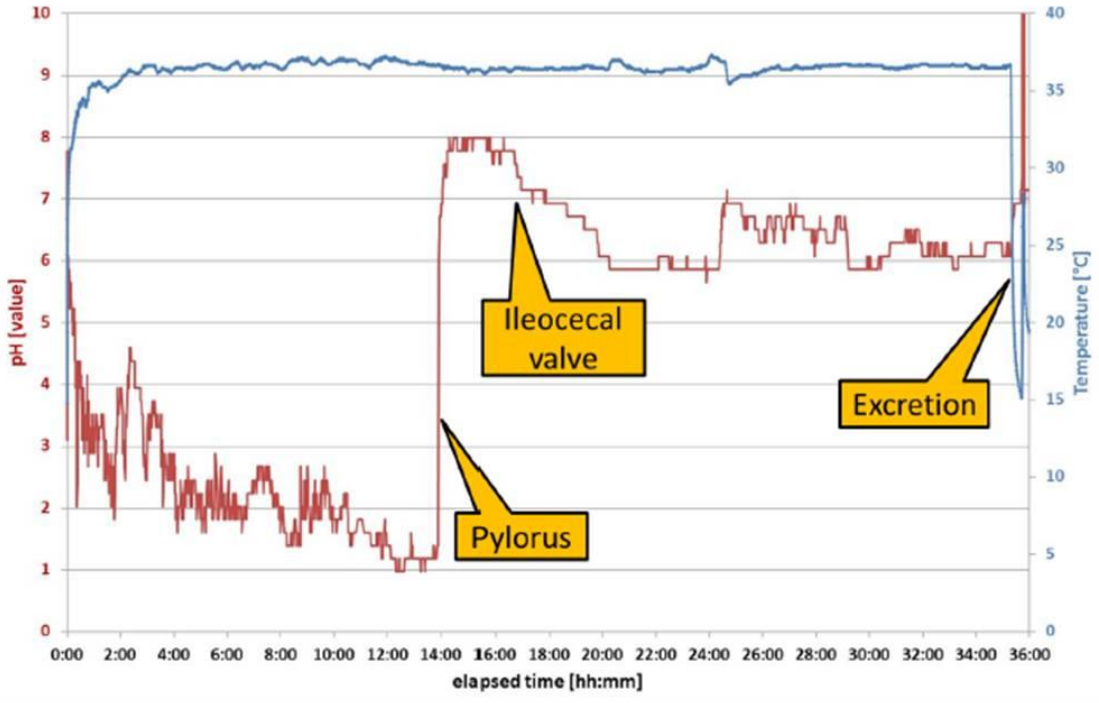
Dog 1 Coarse diet



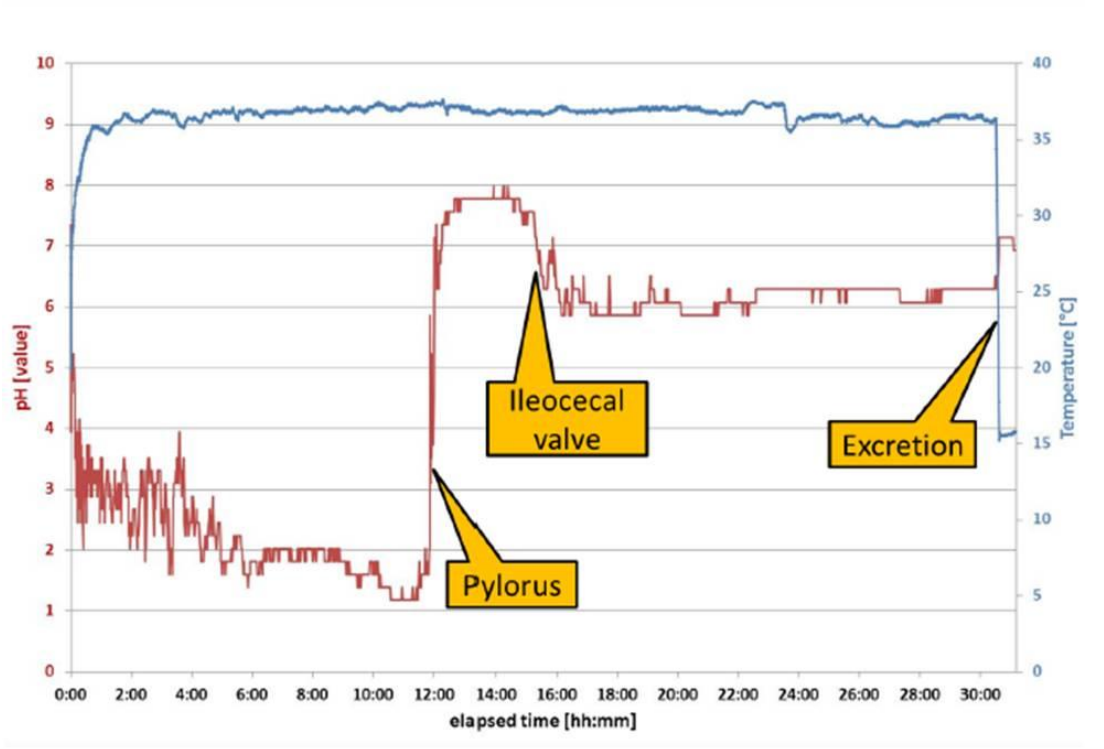
Dog 2 Fine diet



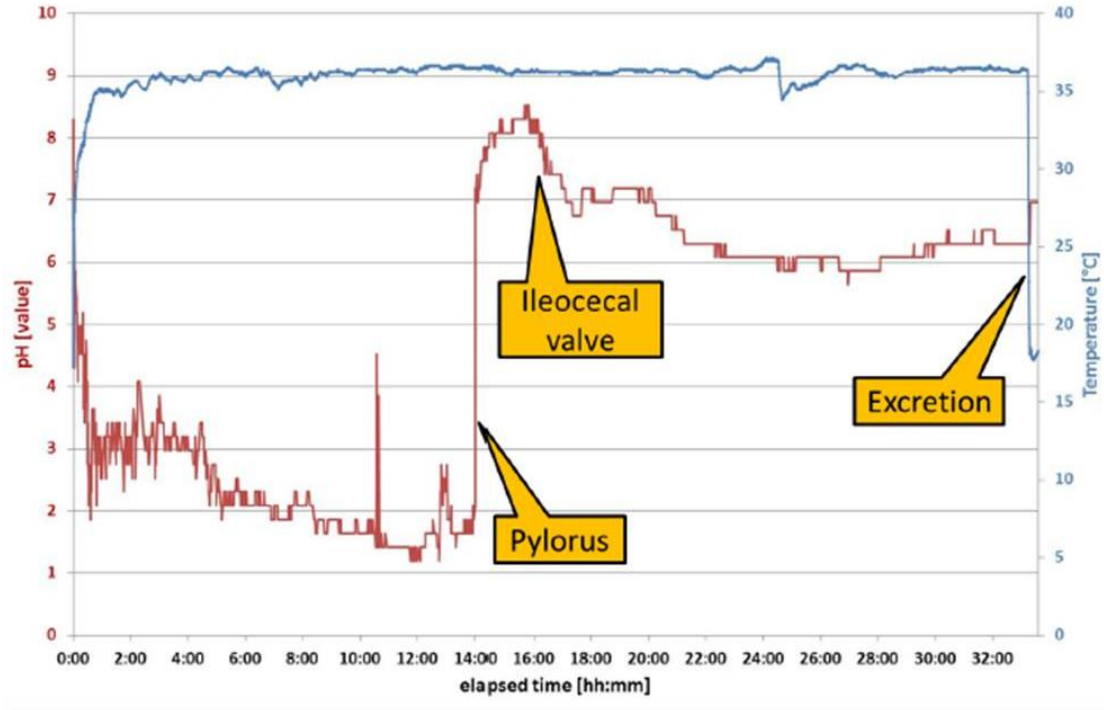
Dog 2 Coarse diet



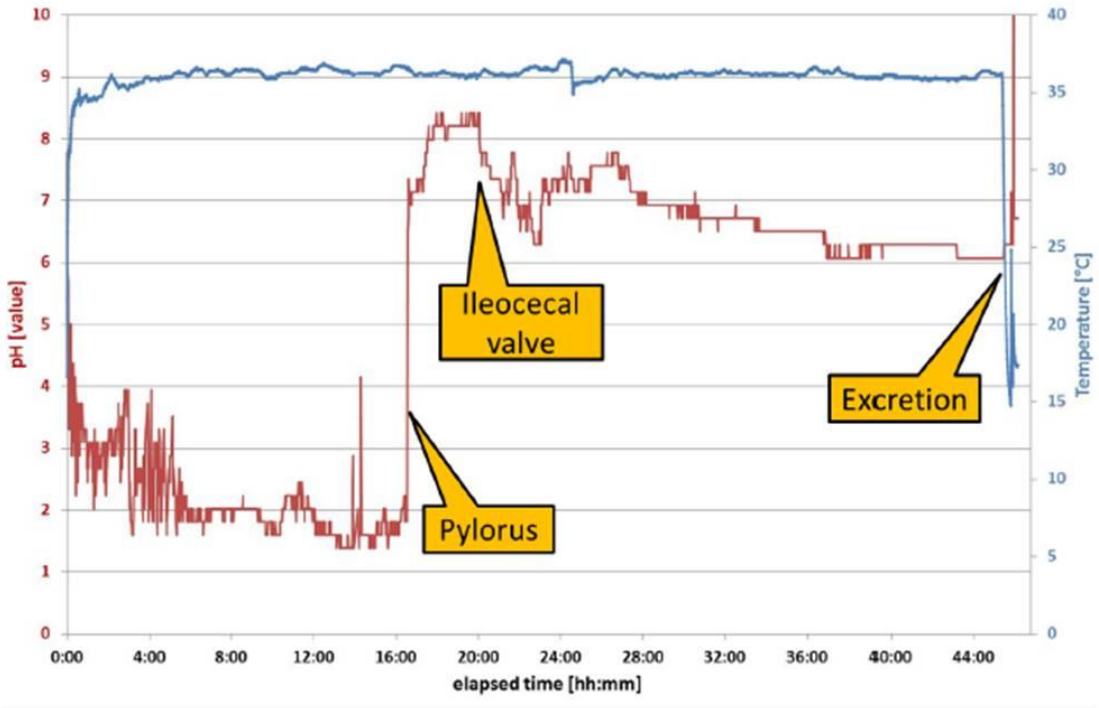
Dog 3 Fine diet



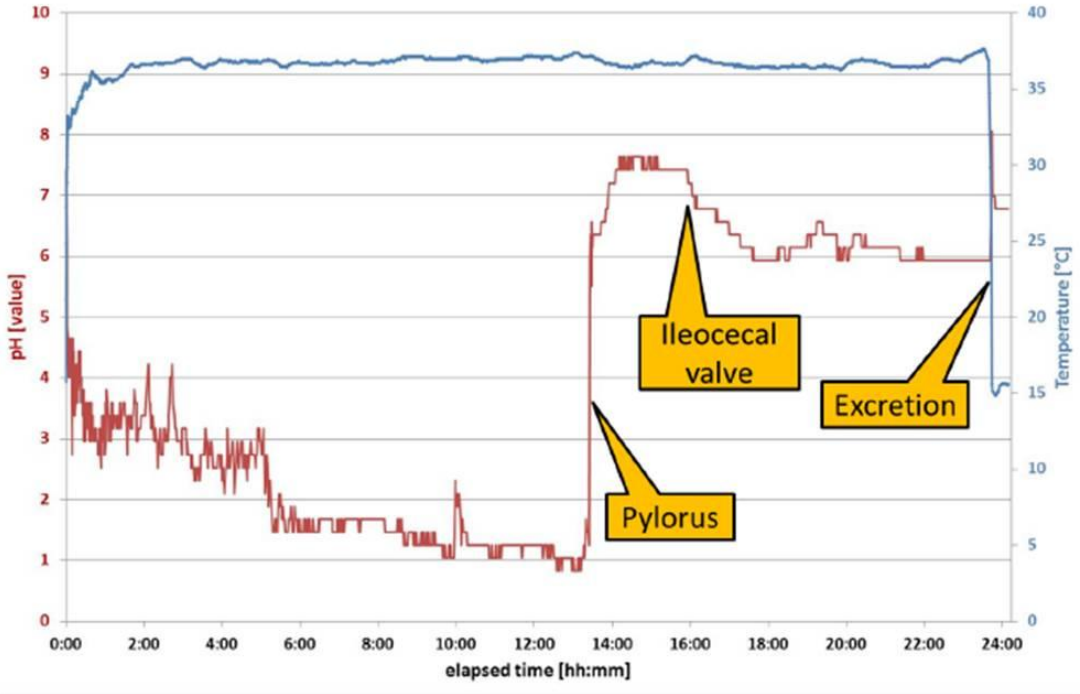
Dog 3 Coarse diet



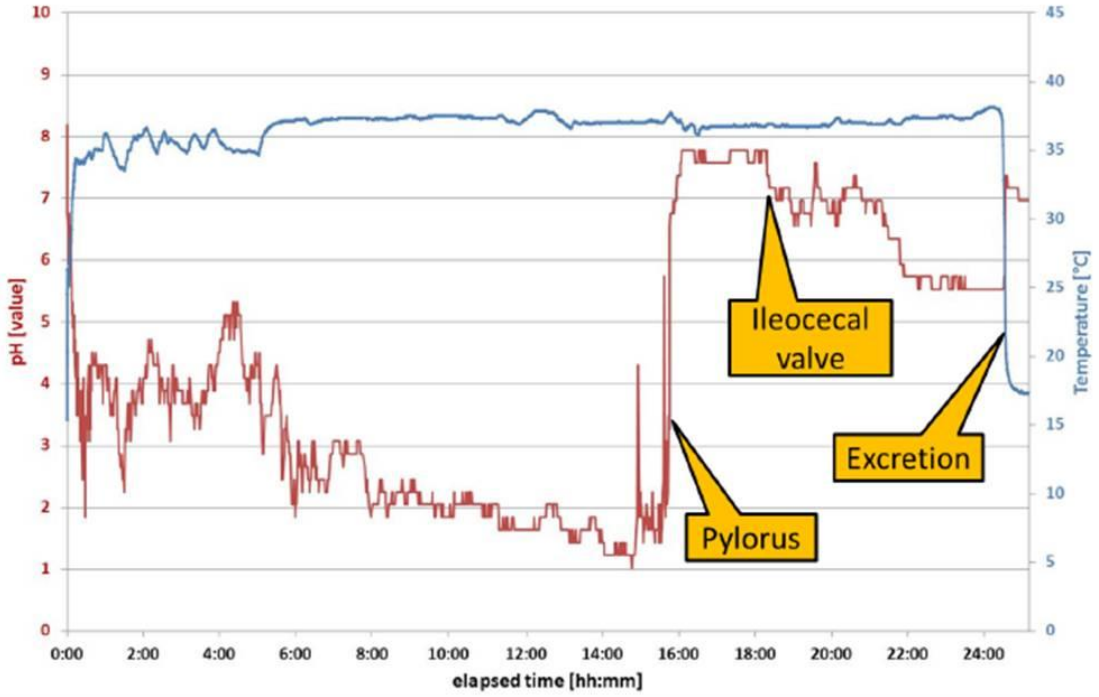
Dog 4 Fine diet



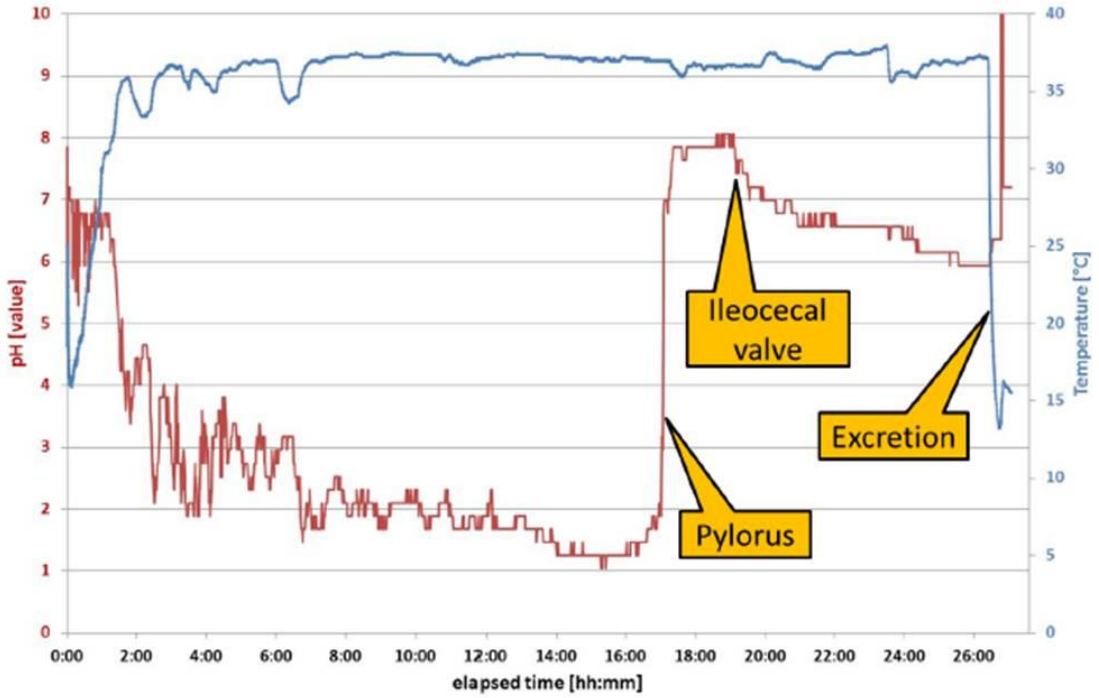
Dog 4 Coarse diet



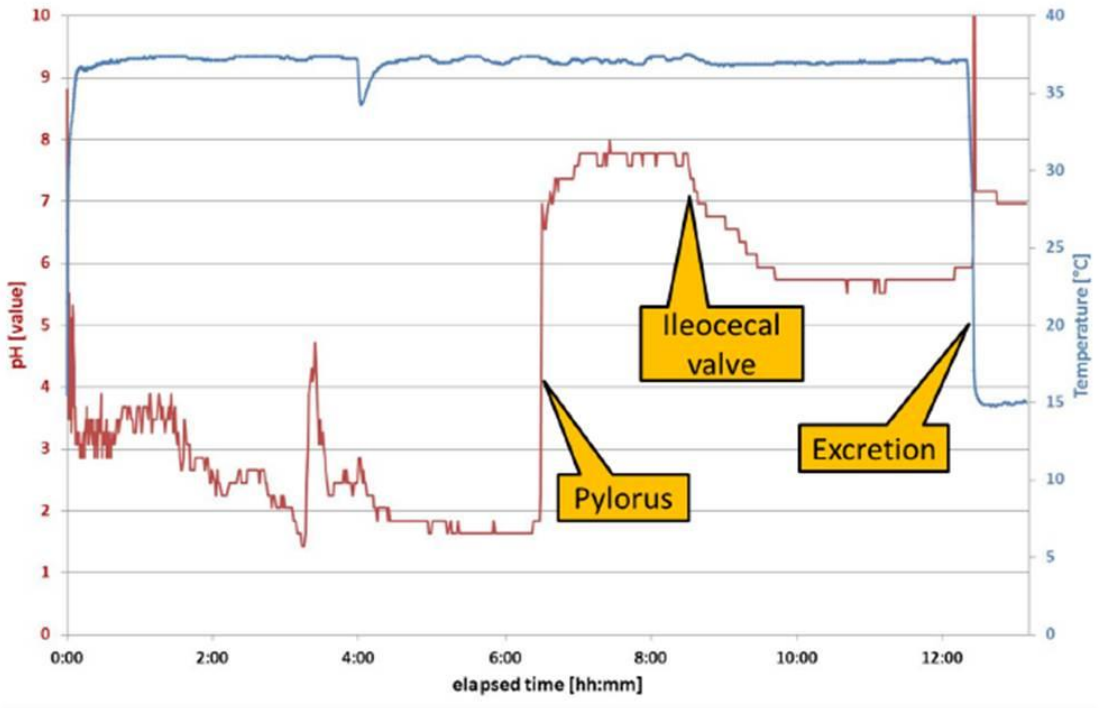
Dog 5 Fine diet



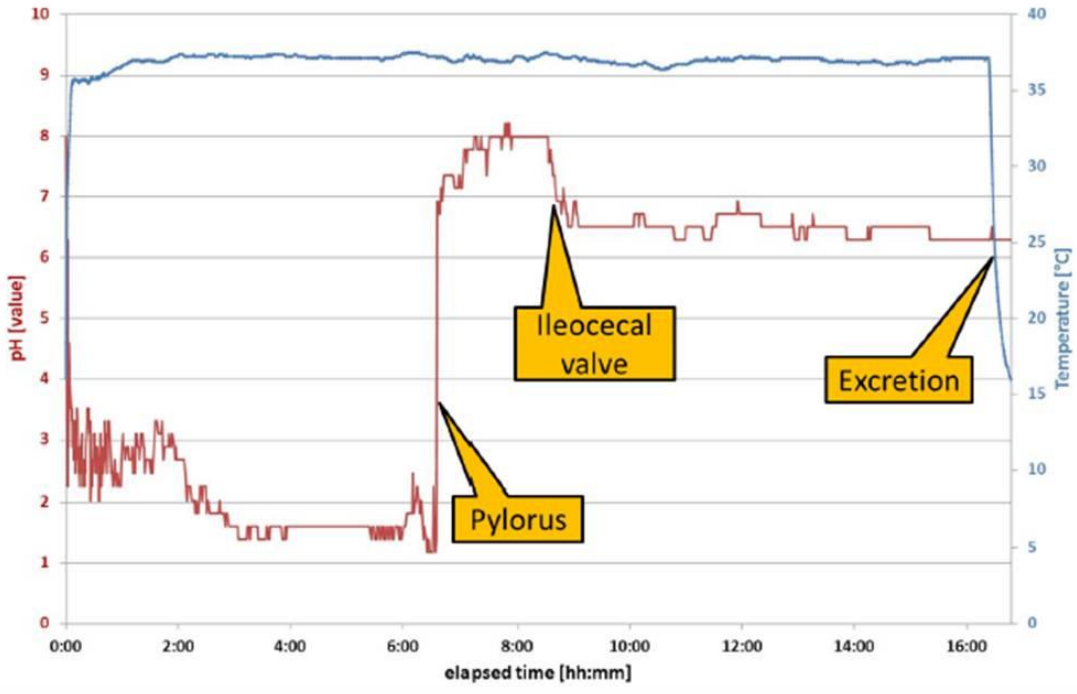
Dog 5 Coarse diet



Dog 6 Fine diet



Dog 6 Coarse diet



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Isabelle, jouw vluchtige bezoekjes aan het labo waren grappig!

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Peace out,
Annelies

Curriculum Vitae

Annelies De Cuyper werd geboren op 7 februari 1989 te Gent. In 2007 behaalde zij haar diploma secundair onderwijs in de richting Wetenschappen-Wiskunde aan het Heilig-Hart College te Halle. In hetzelfde jaar startte zij de studies Diergeneeskunde aan de Universiteit van Gent. Zij behaalde in 2010 haar Bachelor diploma met de grootste onderscheiding en in 2013 haar Master diploma (Optie Onderzoek-Rund) met grote onderscheiding. Zij schreef haar thesis over het kopermetabolisme bij runderen in Ethiopië.

In december 2013 behaalde zij een IWT beurs voor strategisch basisch onderzoek en startte als doctoraatsbursaal aan de vakgroep Voeding, Genetica en Ethologie. Gedurende vier jaar onderzocht zij de verteringsfysiologie van terrestrische carnivore diersoorten met een focus op carnivoor-prooi relaties.

Annelies was promotor en co-promotor van meerdere studenten en is auteur of medeauteur van meerdere wetenschappelijke publicaties en rapporten. Zij presenteerde op meerdere nationale en internationale congressen. Op het 11e congres van de AZA Nutrition Advisory Group (NAG) in Portland (USA) won ze twee jaar geleden de Roy McClements Student Award.

Annelies De Cuyper was born on the 7th of February 1989 in Ghent, Belgium. In 2007, she obtained her diploma of secondary high school in Science-Mathematics at the Heilig-Hart College in Halle. In the same year, she started her studies of Veterinary Medicine at Ghent University. She obtained her Bachelor diploma in 2010 with the greatest distinction and her Master diploma in 2013 with great distinction (Option Research-Cattle). She wrote her master thesis on the copper metabolism of cattle in Ethiopia.

In December 2013, she obtained a scholarship from the Agency for Innovation through Science and Technology (IWT) and started working as a PhD student at the Department of Nutrition, Genetics and Ethology. For four years, she investigated the digestive physiology of terrestrial carnivorous mammals with a strong focus on carnivore-prey relationships.

Annelies supervised several graduate students and has authored and co-authored several publications in international scientific journals. She presented her work at several national and international conferences. In 2015, during the 11th conference of the AZA Nutrition Advisory Group (NAG) in Portland (USA), she won the Roy McClements Student Award.

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